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THE LIFE HISTORY OF COTYLOPHORON COTYLOPHORUM, A TREMATODE FROM RUMINANTS

WITH NINE PLATES

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INTRODUCTION

The knowledge of North American trematode life histories is very limited, and in many instances the life histories which have been described lack completeness. This is particularly true of the amphistomes. Cary (1909) published a life history of *Diplodiscus temperatus* which, as Cort (1915:24-30) pointed out, must be considered erroneous. Krull and Price (1932:1-37) determined experimentally the life history of this same form but omitted a description of the sporocyst. Beaver (1929: 13-22) found and described all of the developmental stages in the life history of *Allassostoma parvum,* with the exception of the sporocyst. However, Beaver did no experimental work except to infest the final host. Krull (1934:171-180) obtained eggs of *Cotylophoron cotylophorum* from Puerto Rico and determined experimentally the life history of this parasite, but he did not describe any of the developmental stages.

Looss (1892:147-167) published the life history of *Diplodiscus subclavatus* (Syn. *Amphistomum subclavatum*) but he did not completely describe the miracidium nor experimentally infest the final host. He also described the miracidium of *Gastrothylax gregarius* (1896:170-177); the developmental stages of *Gastrodiscus aegyptiacus* (pp. 177-185) with the exception of the adult; and the developmental stages of *Paramphistomum cervi* (Syn. *Amphistomum conicum*) (pp. 185-191) with the exception of the adult. Takahashi (1928) described briefly some of the life history stages of *P. cervi.*

There are two methods of attack in solving trematode life history problems. One is to attempt to prove specific identity between cercaria and adult by structural comparison, and the other is to find the relationship experimentally. Several authors have described amphistome cercariae and suggested the possible relationship existing between them and known species of adults, but thus far no one has conclusively demonstrated such a relationship. In the present work the experimental method was used and all of the developmental stages were studied successively. Eggs secured from adult worms were hatched and the intermediate snail host was determined by exposing many species of snails to the free-swimming miracidia. The life history stages consisting of the egg and its development, the mature free-living miracidium, the infestation of the intermediate host, the sporocyst, the redia, the cercaria, the metacercaria, the infestation of the final host, and the development of the parasite to sexual maturity in the final host are discussed.

An attempt is made to evaluate the diagnostic value of certain morphological features which have been considered of no specific value by recent writers in extensive revisions of the classification of the amphistomes.

This report constitutes the first complete study of an amphistome life history and the first report of a representative of the genus Cotylophoron from the mainland of North America.

MATERIALS AND METHODS

Material for the study of the various stages of the life history of *Cotylophoron cotylophorum* was obtained from the two kinds of host of this parasite. Mature worms were collected from the rumen and immature ones from the duodenum and rumen of cows, *Bos taurus,* slaughtered at the city *abattoir* at Baton Rouge, Louisiana. The intermediate snail hosts, *Fossaria parva* and *F. modicella,* were collected from lakes, ponds, and drainage ditches in the vicinity of Baton Rouge.

Eggs deposited by worms after removal from the final host were studied alive only. Miracidia, sporocysts, rediae, cercariae, immature and mature worms were studied while alive, in toto mounts, and from sectioned material.

Miracidia were studied alive unstained or stained *intra vitam.* The *intra vitam* stains which gave the best results were methylene blue, brilliant cresyl violet, and neutral red. Fleming's osmic acid, Bouin's, or Bouin's modified with urea and chromic acid, and sublimate-acetic solution were the fixatives used but the first two were best for this material. Miracidia were stained in toto mounts with Biondi's haematoxylin and Ehrlich's acid haematoxylin. For sectioned material Ehrlich's acid haematoxylin was used most often.

Sporocysts, rediae, and developing cercariae were dissected from snails for study while alive and from toto mounts. For sectioning, the entire snail was fixed in warmed Bouin's fixative unmodified or modified with urea and chromic acid. Sublimate-acetic and modified Bouin's were used in fixing specimens for toto mounts. The stains used most often in preparing toto mounts were borax carmine, alum cochineal, and Ehrlich's acid haematoxylin. For sections the latter stain was used almost exclusively with alcoholic eosin as a counter stain.

The mature cercariae were studied alive, in toto mounts and from sectioned material. Hot sublimate-acetic solution and Bouin's were used as fixatives, but the fixed cercariae were always greatly contracted. Alum cochineal and borax carmine gave good results for toto mounts, and Ehrlich's acid haematoxylin for sections. Metacercariae were studied alive only.

Immature and mature worms are extremely resistant to external conditions and become relaxed in cold water only after several hours. The mature worms remain active from 6 to 8 hours, and the young specimens

sometimes are active after 24 hours. When relaxed the worms were placed in warmed sublimate-acetic solution or Bouin's fixative. For toto mounts borax carmine and alum cochineal gave good results, while Ehrlich's acid haematoxylin, Delafield's haematoxylin, and Mallory's triple connective tissue stain followed by eosin gave excellent results in staining sectioned worms.

The final host, *Bos taurus,* was infested by feeding metacercariae encysted on lettuce. The rate of development and the location of the parasites in the body were determined by killing and examining the hosts.

HISTORY OF THE GENUS COTYLOPHORON

Cotylophoron cotylophorum (Fischoeder, 1901) Stiles and Goldberger, 1910 was described by Fischoeder (1901:370) as *Paramphistomum cotylophorum.* His brief description is as follows:

> Nur 5-8 mm lang, gedrungen, dorsoventral schwach abgeflacht. Oesophagus stark musculös. Scharf abgegrenzter Genitalnapf. Hoden fast neben einander.

He places this species in the family Paramphistomidae Fischoeder, 1901 and in the subfamily Paramphistominae Fischoeder, 1901. Later (1903: 546-550) he redescribes this species in much greater detail.

Stiles and Goldberger (1901:15) raised the family Paramphistomidae to the rank of superfamily Paramphistomoidea, having practically the same characteristics as Paramphistomidae Fischoeder. The superfamily they divide into three families, the Gastrodiscidae Stiles and Goldberger, 1910, the Gastrothylacidae Stiles and Goldberger, 1910, and the Paramphistomidae. *Paramphistomum cotylophorum* Fischoeder, 1901 was designated by Stiles and Goldberger (1910:63) as the type species of their new genus Cotylophoron. They distinguish the genus Cotylophoron from Paramphistomum by a single character, the presence of a genital sucker. Fukui (1929:309) in his work on Japanese Amphistomata considers this difference not important enough to be of generic value and wishes to preserve Cotylophoron as a subgenus of Paramphistomum. I agree with Fukui and believe that Cotylophoron should be preserved as a subgenus only. On the other hand, Maplestone (1923:151) and Stunkard (1925: 141) consider Cotylophoron as a distinct genus.

Stiles and Goldberger (1910:63) described a second species for the genus Cotylophoron which they designated as *C. indicum.* The similarity of *C. indicum* and *C. cotylophorum* is evident from the summation of the differences between the two forms by these writers. Their statement is as follows:

> *Cotylophoron indicum* comes close to *C. cotylophorum,* from which it differs chiefly in the structure of the oesophagus, which is provided with a bulbus thicken-

TABLE 1.—SHOWING GEOGRAPHIC DISTRIBUTION OF *Cotylophoron cotylophorum*

Host	Location	Locality of host	Author and date
Bos taurus	?	Togo, German East Africa	Fischoeder 1901
Bos zebu	?	German East Africa	Fischoeder 1901
Bos taurus indicus	Stomach	East Africa	Fischoeder 1903
Ovis aries (sheep)	Stomach	India	Stiles and Goldberger 1910
"Bullock"	Stomach	Sierra Loone, West Africa	Maplestone 1923
Bubalus sp. (buffalo)	Stomach	Nyasaland	Maplestone 1923
Aepyceros melampus (nswala)	Stomach	Nyasaland	Maplestone 1923
"Pagan dwarf bull"	Stomach	Ilorin, Northern Nigeria	Maplestone 1923
Cobus sp. (waterbuck)	Stomach	Zeref, Khartoum	Maplestone 1923
Bubalus sp. (hartebeest)	Stomach	Nyasaland	Maplestone 1923
"Antelope"	Stomach	Nyasaland	Maplestone 1923
"Antelope"	Stomach	Rhodesia	Maplestone 1923
"Domestic cow"	Stomach	Faradje, Belgian Congo	Stunkard 1929
"Domestic calf"	Stomach	Belgian Congo	Stunkard 1929
Neotragus pygmneus (antelope)	Stomach	Medje, Belgian Congo	Stunkard 1929
Adenota kob alurae (antelope)	Stomach	Faradje, Belgian Congo	Stunkard 1929
"Sheep"	Rumen	Vryheid district, Natal	Le Roux 1930
"Goat"	Rumen	Rangoon	Le Roux 1930
"Sheep"	Rumen		
	Duodenum		
	Intestine	Onderstepoort, Africa	Le Roux 1930
"Cattle"	Intestine		
	Duodenum		
	Rumen	Onderstepoort, Africa	Le Roux 1930
"African buffalo"	?	Zululand, Africa	Le Roux 1930
"Cattle"	Rumen		
	Duodenum		
	Intestine	Zululand, Africa	Le Roux 1930
?	?	Puerto Rico	Krull 1932
Bos taurus (domestic cow)	Duodenum		
	Rumen	Baton Rouge, Louisiana	Bennett 1936
Ovis aries (sheep)	Rumen	Baton Rouge, Louisiana	Bennett 1936

ing in the latter species but is without it in the former. The two differ also in details of structure of the copulatory apparatus and in the position of the genital pore. In *C. indicum* the genital sucker is less sharply delimited, projects less, has a much smaller genital atrium, and the genital pore is decidedly postbifurcal . . . .

Maplestone (1923:152-153) has pointed out that the course and length of the esophagus and the size of the esophageal thickening or bulb are subject to considerable variation in *C. cotylophorum;* and that the position of the genital pore varies in relation to the intestinal bifurcation to such an extent that neither of these points is reliable for distinguishing between *C. indicum* and *C. cotylophorum.* He further points out (p. 155) the variability in the size and appearance of the genital atrium in *C. cotylophorum* and states that the shape and number of chambers in this structure cannot be regarded as of any value for specific diagnosis. He concludes that Stiles and Goldberger were in all probability dealing with immature specimens of *C. cotylophorum.* His conclusion (p. 195) as to the diagnostic value of the copulatory structures in other amphistomes is given as follows:

. . . . the presence or absence of a prominent genital papilla, or a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance (*Gastrodiscoides hominis*) than in any other species of the group Amphistomata.

Stunkard (1925:138) attributes Maplestone's viewpoint to a confusion between physiological variations due to degrees and states of functional activity and true structural differences. On the other hand, Fukui (1929: 270) agrees with Maplestone that the shape of the atrium is highly variable according to the protrusion of the genital papilla and so cannot be used for diagnosis. He (p. 319) definitely considers *C. indicum* to be a synonym of *C. cotylophorum.* I am of the opinion that the conclusions of Maplestone and Fukui concerning the importance of the genital apparatus are unsound for reasons to be pointed out later in the discussion of growth changes of *C. cotylophorum* in the final host. However, Maplestone's conclusions concerning the position of the genital pore in regard to the intestinal bifurcation and the variability in the size of the esophageal bulb are correct.

Leiper (1910:244-248) described two new species of trematodes, *Paramphistomum minutum* and *P. sellsi,* from the hippopotamus, which according to Maplestone (1923:158) should be placed in the genus Cotylophoron. Maplestone considers *C. minutum* and *C. sellsi* to be identical. Stunkard (1925:139) and Fukui (1929:307) accept both of them as valid species of the genus because of the large genital sucker in these forms. Regarding the validity of these two species Stunkard states:

According to the description of Leiper, *C. sellsi* is more than twice as large as *C. minutum,* the testes and ovary are about four times as large, whereas the

oral and ventral suckers are actually smaller than those of *C. minutum.* It seems incredible that these differences are mere variations and therefore I am in agreement with Leiper in regarding the two forms as distinct species.

I am of the opinion that Stunkard and Fukui are correct in considering these two species as distinct.

Only three accepted species, *C. cotylophorum, C. minutum,* and *C. sellsi,* have been described for the genus Cotylophoron.

C. cotylophorum is widely distributed as indicated by reports of this parasite from Africa, India, Puerto Rico, and the United States (present paper). The hosts from which it has been reported, its position in the host, the localities from which the hosts came, the names of the authorities reporting the parasite, and the dates of the reports are given in Table 1.

EGG

Appearance and Structure.—The eggs of *C. cotylophorum* are remarkably uniform in appearance. The shape is nearly ovoid, there being a slight attenuation at the opercular end. However, variations occur in which the eggs are completely ovoid or are more distinctly attenuated, giving a pyriform shape to the eggs (Figs. 1-11). The only marking on the shell is a small projection opposite the opercular end. This marking is usually asymetrical in position. The operculum, which measures 22 by 3 μ, articulates with the shell by means of numerous small tooth-like projections which interdigitate with similar structures on the shell.

The eggshell is whitish when seen with the unaided eye but is transparent when seen with the microscope. In optical sections the shell is seen to be variable in thickness, being 2 μ at the operculum, 1.5 μ in the lateral areas, and 3 μ at the posterior end.

When deposited each egg contains from 40 to 50 yolk masses. Each mass is composed of a membranous envelope which is filled with a translucent liquid and numerous small granules. This material imparts to the egg its brownish-yellow appearance when studied under the microscope. The ovum, which is completely enclosed by the yolk cells, is located slightly anterior to the middle of the egg. Cleavage has not occurred in the majority of the eggs when deposited, but in some it will have advanced as far as the second cleavage stage. The outline of the ovum is easily seen in these eggs, although it is completely embedded in yolk, as is the developing embryo.

Size.—The size of the egg is of special interest because many authors attach specific significance to the size of the eggs, based on the extreme limits. Fischoeder (1903:550) in his description of *Cotylophoron cotylophorum* gave the egg sizes as 125 to 135 μ long by 65 to 68 μ wide. Stiles

and Goldberger (1910), Maplestone (1923), and Stunkard (1929) redescribed the worm but did not give the egg sizes. Krull (1934:178) found the average measurement of 12 eggs teased from a preserved specimen to be 126 by 61 μ and the average measurement of 12 eggs collected from the faeces of an infested calf to be 132 by 68 μ.

Hundreds of eggs from many different specimens were measured

TABLE 2.—EGG MEASUREMENTS FOR *Cotylophoron cotylophorum*

Size of worm in mm...	3.7 x 1.3	4.5 x 2.2	6.0 x 2.0	6.6 x 2.5	9.0 x 3.0	9.0 x 3.5
Size of eggs in microns:						
1.	121 x 58	116 x 67	126 x 62	139 x 63	134 x 63	134 x 67
2.	125 x 67	120 x 67	116 x 67	118 x 67	122 x 67	134 x 67
3.	125 x 67	120 x 67	125 x 67	126 x 67	126 x 67	139 x 67
4.	134 x 67	125 x 67	125 x 71	130 x 67	126 x 67	143 x 67
5.	115 x 68	120 x 71	125 x 71	139 x 67	130 x 67	143 x 67
6.	137 x 68	126 x 71	125 x 71	147 x 67	134 x 60	143 x 67
7.	137 x 68	125 x 73	125 x 71	122 x 71	118 x 71	147 x 67
8.	116 x 71	125 x 73	129 x 71	126 x 71	122 x 71	130 x 76
9.	138 x 71	125 x 76	129 x 71	130 x 71	126 x 71	130 x 76
10.	125 x 76	125 x 76	133 x 71	126 x 71	118 x 71	134 x 76
Average size of eggs.	127 x 68	122 x 69	129 x 69	131 x 68	126 x 68	138 x 70

during this study and the variability was found to be much greater than that indicated by Fischoeder or Krull. Extreme variations in size are rare, but eggs as small as 105 by 55 μ and as large as 155 by 76 μ were found. The size of 100 eggs deposited by worms over 6 mm in length varied from 113 to 143 μ in length by 66 to 76 μ in width. The size variation in these eggs which were deposited by worms that had been mature for several months was 30 μ in length and 10 μ in width. The average size of these eggs was 134 by 69 μ.

Table 2 presents data on the size of eggs produced by small, medium, and large individuals. The eggs produced by the two smaller worms averaged 124.5 by 68.5 μ and were much more variable in size than the eggs produced by either the medium or large worms. The average size of the eggs produced by the medium-sized worms was 130 by 68.5 μ while that for the largest worms was 132 by 69 μ. It is possible to conclude from these data that the average size of eggs produced by young worms is less than that of older ones and that egg sizes tend to become more uniform as age increases.

Further study of Table 2 indicates that individuals tend to produce, on an average, either small or large eggs but not both. The smallest worm, shown in column 1, produced eggs slightly larger than the worm shown in column 5. Their bodies were 3.7 by 1.3 mm and 9.0 by 3.0 mm

respectively. On the other hand, the largest worm (column 6), which measured 9.0 by 3.5 mm, produced the largest eggs, and one of the smallest worms (column 2), which measured 4.5 by 2.2 mm, produced the smallest eggs.

The average size of all these eggs in Table 2 is 129 by 68 μ, which is only a little less than that of the eggs from worms which were on an average of much larger size. This tends to support the statement that individuals produce either small or large eggs but not both.

The extreme range of variation for this group of eggs is 32 μ in length and 18 μ in width. Then by taking the extremes shown in Table 2 the egg size for this species is found to be from 115 to 147 μ long by 58 to 76 μ wide, while the average size of the eggs is 129 by 68 μ. This is approximately the same as the averages given by both Fischoeder and Krull. The slightly smaller average is possibly due to the inclusion of measurements made on eggs produced by very small worms.

MIRACIDIUM

Development

The development of the miracidium has been described for very few trematodes, and the descriptions which have been made vary considerably in their completeness. An accurate description of this stage in trematode development is comparatively difficult because of the minuteness and indefiniteness of the miracidial organs. Such a study necessitates both living and fixed materials which must be studied at very short intervals to determine the embryological sequence of organ development. Living material is sometimes difficult to study because of the opaque shell or the enclosed vitelline mass. Fixed materials are also difficult to study since this involves the fixation of embryos at known stages of development, sectioning and staining, followed by intensive study of the material under high magnification. Consequently only a few authors have attempted to describe this stage in the life history of trematodes.

The most complete studies of this nature were made by Thomas (1883) on *Fasciola hepatica;* Looss (1892) on *Diplodiscus subclavatus;* Looss (1896) on *Gastrothylax gregarius, Gastrodiscus aegyptiacus,* and *Paramphistomum cervi;* Ortmann (1908) on *Fasciola hepatica;* Johnson (1920) on *Echinostoma revolutum;* Stunkard (1923) on undetermined species of Spirorchis; Barlow (1925) and Ishii (1934) on *Fasciolopsis buski;* and Suzuki (1931) on *Fasciola hepatica.* Of these workers, Ortmann and Ishii used sectioned and living material while the others made their studies from living material only.

The similarity of the results obtained by these authors as to the se-

quence of organ development is remarkable. Quite naturally, however, the time of appearance of organs varies considerably because of the difference in time required for the miracida to develop under natural or experimental conditions. The slight variations found to occur in the sequence of organ development in these different species of trematodes may have several explanations: first, the difficulty with which such minute structures are recognized in either living or sectioned material; second, the almost simultaneous appearance of some organs; and, third, the lack of accurate, detailed observation.

In the present work, the development of the miracidium of *C. cotylophorum* was studied in living material only. The eggs used in making these observations were secured by taking adult worms from the host and placing them in dishes of water where they would deposit eggs for several hours. The worms were removed before they died and the water was decanted. The eggs were then washed in several changes of water in order to remove as much débris as possible. It was found that if any animal tissues were left in the dishes bacteria would destroy a large percentage of the eggs within a few days. This was demonstrated by allowing the eggs and worms to remain in the same dish until the worms had begun to decompose. In such instances only about ten per cent of the eggs would reach the hatching stage. In order to secure the highest percentages of hatching it was found necessary to change the water on the eggs at least twice each day. When the eggs were thus properly cared for about ninety per cent of them would hatch.

Time Required for Hatching.—Many eggs were obtained throughout the period from August 12, 1933, to June 22, 1934, and a record was kept as to the minimum time required for hatching (Table 3). The eggs secured between August 12, 1933, and January 4, 1934, were kept at laboratory temperatures, but the time required for the eggs to hatch increased as the winter advanced, due to the fact that laboratory temperatures fluctuated with outside temperatures, except during the day when the laboratory was heated. Between January 6 and February 23 the eggs obtained were subjected to outside temperatures at all times and none of these eggs hatched. The eggs obtained between March 28 and June 26 were again kept at laboratory temperatures, but throughout this period laboratory temperatures fluctuated day and night with outside temperatures.

No controls were established or temperature records kept for these egg-hatching experiments but the data indicate that temperature conditions are of primary importance in determining the time required for the eggs to hatch. The time required steadily increased from 15 to 29 days as the temperatures became lower from August 12, 1933, until February

23, 1934. During the period from January 6 to February 23 no eggs reached the hatching stage when exposed to outside temperatures due, it is believed, to the fact that freezing temperatures occurred a number of times. The eggs in this experiment would begin to develop, some developing as far as the ciliated stage. None was observed which had

TABLE 3.—RESULTS OF EGG-HATCHING EXPERIMENTS
Showing the minimum time required for eggs of *Cotylophoron cotylophorum* to hatch at different times of the year

Deposited	Hatched	Days elapsed	Deposited	Hatched	Days elapsed
Aug. 12	Aug. 27	15	Jan. 26	none hatched	
Aug. 17	Aug. 30	13	Feb. 1	none hatched	
Aug. 18	Sept. 1	14	Feb. 6	none hatched	
Aug. 29	Sept. 10	19	Feb. 13	none hatched	
Aug. 31	Sept. 21	22	Feb. 20	none hatched	
Oct. 23	Nov. 20	28	Feb. 23	none hatched	
Oct. 31	Nov. 22	23	Mar. 28	Apr. 24	27
Nov. 1	Nov. 23	24	Apr. 4	Apr. 25	21
Nov. 8	Dec. 2	24	Apr. 6	Apr. 27	21
Nov. 15	Dec. 14	29	Apr. 9	May 2	23
Nov. 24	Dec. 20	26	Apr. 17	May 9	22
Dec. 6	Dec. 31	25	Apr. 20	May 11	21
Dec. 20	Jan. 14	28	Apr. 26	May 16	20
Jan. 3	Feb. 1	29	May 29	June 15	18
Jan. 4	Feb. 2	29	June 8	June 20	12
Jan. 6	none hatched		June 22	July 3	11
Jan. 19	none hatched		June 26	July 8	11
Jan. 24	none hatched				
			Average Time Elapsed		21

developed beyond this condition, although living embryos were found as long as 35 days after the beginning of the experiment. The time required for the eggs to hatch during the period from March 28 to July 8 decreased from 27 days at the beginning to 11 days at the end of the experiments. This decrease in time follows steadily the increase in temperature.

In this series of experiments the time required for the eggs to hatch varied from 11 days during one of the warmest periods of the year to 29 days during one of the coolest periods, even though the eggs were kept at laboratory temperatures. The average time required for all the eggs to hatch in this series of experiments, which is overbalanced in number in the cooler months, is 21 days. In those experiments in which no eggs reached the hatching stage the temperature dropped below freezing for a part of the time. Consequently, the conclusions which may be drawn are that temperature is of great importance in determining the time required for the eggs to hatch and that freezing temperatures are fatal to them.

Developmental Rate.—A study of the rate of development of *C. cotylophorum* miracidia was made on many of the eggs used in the hatching experiments, and the results of those experiments show that the developmental rate is influenced directly by temperature. However, only the minimum time required for hatching is given in Table 3. The majority of the eggs will hatch within a few days after the minimum time but there are others which do not complete their development until months afterwards. One egg culture in which hatching began on January 14 was kept in order to determine the time required for all of the eggs to hatch.

TABLE 4.—DEVELOPMENTAL RATE OF MIRACIDIA OF *Cotylophoron cotylophorum*
Based on measurements (in microns) of different individuals at four-day intervals

No.	April 4	April 8	April 12	April 16	April 20	April 25
1	Diameter of ovum 18 to 25	25 x 25	55 x 38	90 x 43	154 x 38	169 x 29
2		25 x 25	55 x 42	65 x 46	160 x 38	153 x 32
3		25 x 25	59 x 42	69 x 46	101 x 42	189 x 34
4		25 x 25	59 x 42	101 x 46	105 x 42	197 x 34
5		25 x 25	59 x 42	79 x 47	118 x 42	189 x 36
6		29 x 29	63 x 42	90 x 47	143 x 42	168 x 38
7		29 x 29	50 x 46	97 x 47	103 x 46	182 x 38
8		29 x 29	59 x 46	72 x 49	113 x 46	193 x 38
9		29 x 29	71 x 46	72 x 49	105 x 50	180 x 42
10		34 x 34	62 x 50	80 x 50	55 x 55	210 x 63
Average		28 x 26	59 x 44	82 x 47	116 x 44	184 x 39

This culture was examined from time to time and after an interval of five months occasional developing embryos could be found. Other cultures were kept for varying lengths of time and this condition was observed in all of them. The cause of this slow development was not determined.

The seemingly inherent variation in the rate of development and that induced by changing temperature makes the age of the embryo an unreliable standard for determining the time of appearance of the structures of the fully developed miracidium. However, if the age and size of a sufficiently large number of embryos of any developmental series are combined a fairly reliable standard is obtained. This method was used in preference to following the development of one embryo. In this way the average rate of development can be determined, and at the same time the appearance of organs can be correlated with the age and size of any one individual.

While the developmental rate of the miracidium was studied in many eggs, only the results of observations made on one developmental series will be discussed. This series represents the average minimum time of development required by all of the eggs obtained. In making this study the eggs were observed when deposited, and cleavage was followed for

TABLE 5.—DATA ON APPEARANCE OF STRUCTURES DURING DEVELOPMENT OF MIRACIDIA OF *Cotylophoron cotylophorum*

Size in microns	Cilia	Flame cells	Primitive gut	Movement	Brain	Germ balls	Apical papilla	Penetration glands
72 x 49	not dev.	not dev.	not dev.	none	not dev.	not dev.	not dev.	not dev.
76 x 46	developed	not dev.	not dev.	none	not dev.	not dev.	not dev.	not dev.
80 x 46	developed	developed	developed	none	not dev.	not dev.	not dev.	not dev.
85 x 43	developed	developed	developed	infrequent	not dev.	not dev.	developed	not dev.
105 x 46	developed	developed	developed	frequent	?	?	developed	not dev.
122 x 46	developed	developed	developed	frequent	developed	developed	developed	developed

several hours. The eggs were then studied at four-day intervals until hatching began. Measurements were made on ten embryos selected at random at the end of these intervals in order to obtain data on the rate of growth. In addition, at the end of each interval many embryos were studied in an attempt to determine at what size and age the various organs of the miracidium made their appearance.

The data obtained on growth rate and the size at which organs can first be recognized in the embryo are presented in Tables 4 and 5.

From Deposition to End of Fourth Day

Cleavage.—Eggs are usually deposited before cleavage begins, but occasionally they are deposited as far advanced in cleavage as the four-cell stage. The ovum or the embryo is usually situated slightly anterior to the middle of the egg, entirely surrounded by the vitelline cells, although it is sometimes peripheral in position.

The early stages of cleavage in various trematodes were described as being unequal by Ortmann (1908) and Ishii (1934). Thomas (1883) and Johnson (1920) do not describe cleavage, but their figures show that the early cleavage stages result in cells of equal size. Suzuki (1931) figures cleavage as being very regular through all of the early stages. Looss (1896) and Barlow (1925) discuss the early cleavage stages but neither their discussions nor figures throw any light on the subject. Stunkard (1923) does not mention these early stages.

The first cleavage of the ovum in this species results in two cells slightly unequal in size. As a result of the second cleavage there are one large and three small cells, one of which is considerably larger than the other two (Fig. 12). These stages occur in most of the eggs 12 hours after being deposited, but some have advanced to the eight-cell stage at the end of this time. It was impossible to determine accurately the size of the cells in the eight-cell stage due to the surrounding vitelline cells.

Size of Embryo.—The increase in size of the embryo is slight during the first four-day period of incubation. The size of 10 embryos at the end of this period is given in Table 4, column 2. At this age and size the embryo appears as a rounded, semi-transparent ball of cells, in which the nuclei vary from 3 to 5 μ in diameter. It is clearly delimited from the enclosing yolk material.

Four to Eight Days

Size.—There is a marked increase in size at the end of the second four-day period, as indicated in Table 4, column 3. Some of the embryos have reached their maximum width but none of them have structures

which could be identified with certainty, other than the nuclei mentioned as being present in the earlier stages.

Vitelline Cells.—Up to this point in development there is very little change in the appearance of the vitelline cells. The original outlines of the cells are still distinct but there are fewer granules in them, giving them a more hyaline appearance.

Eight to Twelve Days

Size.—Between the eighth and twelfth days a difference in rate of development becomes very evident. The largest embryo observed on the twelfth day measured 101 by 42 μ while the smallest measured 65 by 46 μ, which is somewhat smaller than the largest embryo recorded at the end of the eighth day. However, the average rate of increase in size is comparable to that of the second four-day period. The majority of the embryos have reached their maximum width by the twelfth day and many structures have made their appearance.

Cilia.—The cilia are the first structures developed which can be definitely recognized, being seen on an embryo which measured 76 by 46 μ (Table 5; Fig. 5). The size of the embryos on the twelfth day (Table 4, column 4) indicates that many of them possessed cilia on the ninth or tenth day of development while there were some which had not developed them by the twelfth day.

The presence of the cilia is evidence that the epithelial cells have developed in an earlier stage but neither the nuclei nor cell boundaries could be seen at any stage in development, although the anterior and posterior limits of the cells could sometimes be seen in optical section at the lateral limits of the embryo.

Primitive Gut.—Following the cilia in development are the so-called primitive gut and two flame cells which appear at approximately the same time. These structures were first observed in an embryo which measured 80 by 46 μ (Fig. 9). The primitive gut at this stage consists of two large cells filled with granular material similar to that present in the primitive gut of the fully developed miracidium. The cells measure 17 by 11 μ and are located in the center of the body, 9 μ from the anterior end. The nucleus of each cell is 6 μ in diameter and contains a large chromatin knot which is 2 μ in diameter. The flame cells, which are located laterally and slightly posterior to the middle of the body, measure 5 by 3 μ. The ducts leading from them could not be seen.

The exact size of the embryo at which the two primitive gut cells divide to form the four cells characteristic of it in the mature miracidium was not determined but the four-cell condition was found in an individual

which measured 90 by 42 μ (Fig. 2). In this individual the primitive gut had begun to elongate, appearing similar to that in the fully developed miracidium. On the other hand, some much larger individuals (Fig. 10) possessed a gut much less advanced in development. With the appearance of the gut or shortly afterwards the apical papilla can be distinguished as a small, non-ciliated projection at the anterior end.

Muscle Tissue.—The muscle layers of the miracidium were not seen in these early stages but their presence is denoted by movement. In this series no movement was detected in any embryos under 90 by 43 μ, although it was seen in embryos of other series at a size of 85 by 45 μ. Movement in these earlier stages is very infrequent and consists of very slow and slight contractions of the anterior half of the body.

Subepithelial Tissue.—The subepithelial layer (Fig. 9) became distinguishable from the other tissues of the body during the period between the eighth and twelfth days. Not all of the nuclei of this layer can be seen, but some of them can be seen easily in optical section. They may be recognized by their characteristic ovoid shape and their position immediately beneath the ciliated epithelium. The nuclei, which are the only criteria by which this layer may be recognized, measure approximately 5 by 3 μ.

Vitelline Membrane.—According to Ortmann (1908) and Ishii (1934), working on different species of trematodes, the vitelline membrane is formed by cells which break away from the embryo during the early developmental stages and migrate to the periphery of the vitelline mass where they flatten, unite, and eventually enclose the entire vitelline mass and the embryo. In the eggs of this series the vitelline membrane was not clearly distinguishable prior to the twelfth day. Its recognition depends upon its withdrawing from the eggshell at some point, and this point is always the opercular end of the egg (Figs. 2, 3). It is then seen as a very thin membrane. The space left at the opercular end of the egg is filled with a clear liquid at first, but it is finally occupied by a viscid, granular mass called a "mucoid plug" by Barlow (1925).

Vitelline Cells.—There is no marked change in the appearance of the vitelline cells or masses during the earlier developmental stages. There is, however, a gradual decrease in the number and an increase in the size of the masses. Perhaps this is due to the breaking down of the original masses and a subsequent coalescence of the liquids contained in them. There is also a gradual decrease in the number of vitelline granules.

The most extensive changes, in the series under discussion, occurred between the eighth and twelfth days when the vitelline masses were broken down rapidly until there were left only a few relatively large masses. However, there is no uniformity in these changes. The condition

is true for some embryos while others of approximately equal age and size still have a large number of small vitelline masses (cf. Figs. 2 and 11).

The cilia do not break down the yolk masses in this species as they do in *Fasciolopsis buski* Barlow (1925). The cilia are very seldom in motion until late in development and could scarcely be of any service in this respect. However, since they do break down shortly after most of the miracidial structures are present, it is possible that the movements of the embryo and perhaps some secretion produced by the embryo hasten this process.

Nerve Tissue.—The nervous system develops simultaneously with the primitive gut and the flame cells. It consists of many cells located on the dorsal surface of the body between the gut and the flame cells (Figs. 9, 10, 11). The nuclei are the only structures which can be seen distinctly, although there is a clear area ventral to them which probably represents the early stages of the fibrous brain.

Germinal Tissue.—The primordial germ cells can be recognized in embryos as small as 80 by 46 μ. They are massed together in the posterior third of the body, the only distinctive feature being the large nuclei characteristic of these cells. The nuclei measure 4 to 5 μ in diameter and are surrounded by very small amounts of cytoplasm.

Mucoid Plug.—The mucoid plug is not formed until after the embryo has acquired most of its organs. It was first seen in an embryo which measured 90 by 42 μ (Fig. 2), where it appeared as a granular, translucent mass at the opercular end of the egg. Barlow (1925) states that this mass is formed only at the opercular end of the egg and that perhaps it prevents embryonal secretions from loosening the operculum. This is not true in the present species since the plug may develop at either or both ends of the egg (Figs. 3, 6, 7). It becomes so viscid as development proceeds that only by extremely vigorous movements can the miracidium indent it. When present at the anterior end of the egg it forms an effective barrier between the miracidium and the operculum which has to be removed before the miracidium can hatch. I believe that this mucoid plug is nothing more than concentrated waste material excreted by the miracidium. Its positions in the egg and the fact that it does not appear until after the flame cells begin to function tend to support this belief, as does the fact that this plug increases in size and viscosity as the embryo develops. Furthermore, the concentration of this mass outside the vitelline membrane indicates that the membrane is selective and prevents the embryo from being enveloped in its excreta.

Twelve to Sixteen Days

After the twelfth day the only other structures to make their appearance are the four penetration glands which were first seen in an individual 122 by 46 μ (Fig. 11). Between the twelfth and sixteenth days there is a considerable increase in length and a slight decrease in width of the embryo (Table 4, column 5). Some of the embryos become longer than the egg during this period, and the posterior end of the body is flexed to provide for further increase in size (Figs. 3, 4, 6, 7).

The vitelline masses are reduced in number until there are only two large bodies which partially enclose the embryo. These are kept pressed tightly against the embryo by the vitelline membrane but move freely when the embryo moves. A few scattered vitelline granules are still present at the end of this period.

Sixteen to Twenty-one Days

Between the sixteenth and the twenty-first days there was a remarkable increase in growth. The average size of 10 embryos on the sixteenth day was 116 by 44 μ, while the average size of 10 embryos on the twenty-first day was 184 by 39 μ. There was an average decrease of 5 μ in width and an average increase in length of 58 μ. The position of the miracidium in the egg immediately prior to hatching is shown in Fig. 7.

The development of the miracidium as described here coincides in practically every detail with the development of the miracidia described by the workers mentioned earlier in this discussion. This result points to the conclusion that the chronological sequence of organ development in trematode miracidia is essentially the same.

Hatching

The process of hatching in *C. cotylophorum* was found to be more complicated than has been described for most trematode eggs. Barlow (1925), in describing the hatching of the eggs of *Fasciolopsis buski*, states that the glands of the miracidium are of importance in effecting its release. My observations on the present species fully support this view.

No criterion was discovered which would seem to indicate exactly when the miracidium begins its hatching activities, but such efforts continue for approximately 48 hours. The mucoid plug is located at the opercular end of the egg in almost every case and is the first obstacle which has to be removed. This plug may reach a thickness of 34 μ, so crowding the miracidium in the remaining space that its body is bent almost double. No effort was made to follow in detail the formation of the structure, but, as previously stated, it makes its appearance after the

flame cells begin to function, and it is probably formed from waste materials concentrated outside the vitelline membrane. If this is true then the plug would increase in size until the miracidium begins to destroy it. If it were possible to determine at just what moment the miracidium starts to do this then it would be possible to determine at what moment hatching efforts begin.

The plug is removed or destroyed at an extremely slow rate. A number of miracidia were observed for as long as four hours each and in no case did one make any measurable progress through it. However, it is easy to find all stages of destruction of the plug in an egg culture in which miracidia are hatching.

The initial efforts consist of applying the tip of the apical papilla to the flat base of the plug, usually near its center, and then strongly contracting the circular muscles of the body. This gives the impression that the miracidium is attempting to push the plug out of the egg or to one side. It is most probable that such contraction stimulates glandular secretions and aids in forcing out the secretions. The body may contract at any point but usually the strongest contractions occur at the base of the papilla and at the junction of the epithelial plates. During such contractions the ducts of the glands become more prominent than at any other time.

The cilia apparently are of little service in hatching, although sometimes they beat energetically as the miracidium applies its apical papilla to the plug. The short, stiff cilia present on the anterior half of the first tier of epithelial plates seem to serve as a brush. At times the miracidium slightly withdraws the apical papilla and twitches its body from side to side, which may brush off parts of the plug loosened by its secretions. The miracidium is not continuously active, each period of muscular activity being followed by a slightly longer period of quiescence. Neither of these periods exceeds more than two minutes.

The plug is entirely removed after an undetermined length of time, no trace of it being found in eggs in which the miracidium is in contact with the operculum. The apparent necessity for completely removing this plug was demonstrated in one instance. In attempting to orient an egg under a cover slip by tapping gently on it with a needle the operculum was slightly loosened and a drop of water entered the egg. The plug immediately expanded until it filled approximately two-thirds of the egg and crushed the miracidium (Fig. 8). The fact that the operculum does not open as soon as the miracidium comes in contact with it is attributed to the possibility that it is cemented shut by some secretion of either the adult worm or the embryo. Many miracidia which were undergoing strong muscular contractions in an apparent attempt to liberate themselves

were observed for varying lengths of time. Occasionally one would be successful. The operculum springs back allowing water to flow in. This seems to stimulate the miracidium to vigorous activity. However, it takes the miracidium only a few seconds to find the opening and it immediately begins to squirm through. The fact that the operculum remains closed, in some instances for hours, after the miracidium comes in contact with it points directly to the conclusion that it is eventually loosened by glandular secretion and not by intermittent muscular activity.

The opercular opening is considerably less in diameter than the body of the miracidium and it takes miracidia from five seconds to twelve minutes to get out of the egg. The apical papilla, which is narrow, goes through the opening readily but the remainder of the body passes through in some instances only after a prolonged period of incessant activity. Some swim out immediately and some were observed swimming with the eggshell still attached to the posterior end.

The miracidia do not rotate or turn around in the egg under normal conditions but they do so when the opercular opening becomes filled with débris and they are unable to penetrate it. Under such conditions they remain in constant activity until death ensues. In all egg cultures about two per cent of the embryos were found developing in a reversed position. The mucoid plug in all such cases observed was present at the opercular end of the egg, but the hatching efforts of the miracidium in each case were directed against the end opposite the operculum. None was ever observed to turn around.

Mature Miracidium

General Activity.—The miracidia hatch throughout the day and night but the numbers hatched between 8:00 and 11:00 A.M. are so much greater that the hatching may be considered periodic. Between 3:00 and 5:00 P.M. large numbers are hatched also, but relatively much fewer than during the morning hours. They can be induced to hatch in considerable numbers at any time by stirring the egg culture while under a strong light. Their positive phototropism is evidenced by the fact that they always congregate on the lighted side of a container.

After hatching the miracidia are extremely active. They usually swim in a straight line at top speed, but they follow a zigzag course at slower speeds. The body rotates in a counter clockwise direction. At other times it may contract in such a way that the anterior end of the body is pulled to one side and as a result it swims in a small circle.

Shape and Size.—When swimming straight ahead the body is pyriform, the greatest diameter being one-fifth of the body length from the anterior end. From this point the body tapers sharply anteriorly to

terminate in the small blunt apical papilla, while posteriorly the decrease in size is much more gradual, leaving the posterior end bluntly rounded (Fig. 17).

The ability of the miracidium to change its shape is quite marked. At slow rates of speed it swims along alternately contracting and extending its body, and when stopped it may contract to such an extent that it has the appearance of a slightly ovoid ball. When the anterior end is contracted the apical papilla projects from the bottom of a conical depression formed by an invagination of the body. The miracidium very often swims along in this contracted state alternately thrusting out and withdrawing the papilla while the short cilia present at the anterior end are beating rapidly. The initial impression given is that the miracidium is feeding, because particles in the water are swept down into the funnel-shaped cavity toward the tip of the papilla, but nothing was ever observed to enter the gut.

If no host is available to the miracidium it dies after 8 or 10 hours. When near death it becomes greatly distended and moves very slowly. The body becomes vesicular as the tissues begin to break down and the epithelial plates swell away from the body, but the cilia remain active for some time afterward. Motion finally ceases except for a very slow turning around in a small circle, which continues until the epithelial plates break away completely from the body. As the internal tissues break down, the nuclei float free and are concentrated in one or more groups within the body.

General Description.—Descriptions of the morphology of fully developed miracidia have been made for comparatively few species of trematodes and many of these are not complete. In view of the limited number which have been described in detail it is difficult to judge the completeness of any of the present descriptions. The miracidium of *C. cotylophorum* possesses a ciliated epithelial covering, a primitive rhabdocoel gut, penetration glands, an excretory system, reproductive tissue, and a nervous system.

The description of the miracidium of *C. cotylophorum* presented here is based on a study of living specimens unstained and stained *intra vitam*, toto preparations, and sectioned material.

Size.—The exact size of living miracidia is very hard to determine because of their incessant activity. However, an attempt was made to measure them by using the hanging drop method. To prepare a hanging drop, one or two miracidia were pipetted onto a cover slip and the excess water was removed. A few cotton fibers were added to the remaining water. The cilia of the miracidium become entangled in these fibers and hold it in a relatively stable position without distortion of the body. It

continues to contract and extend itself but at intervals it becomes motionless. The size of miracidia measured in this way was found to vary from 153 to 210 μ in length and from 32 to 63 μ in width; the average size of 10 was 164 by 39 μ. An attempt was made to measure them first alive and then fixed, but this was unsuccessful. The vapors of warmed Bouin's and Fleming's fixatives were employed. The contraction of the miracidia fixed in this way was always abnormally great when compared with those

TABLE 6.—MEASUREMENTS OF FIXED MIRACIDIA (IN MICRONS)

No.	Length	Width	No.	Length	Width
1	143	38	14	168	32
2	151	38	15	134	38
3	155	38	16	160	34
4	164	38	17	164	38
5	168	38	18	151	42
6	197	38	19	164	34
7	143	38	20	181	38
8	168	29	21	176	38
9	139	42	22	155	34
10	172	42	23	160	38
11	147	46	24	147	34
12	151	50	25	168	34
13	168	46	*Average*	159.8	38.2

fixed by other methods. The size of 25 miracidia fixed by flooding them with warmed Bouin's fixative is given in Table 6. The range in size of these fixed specimens was 134 to 197 μ in length by 29 to 50 μ in width, with an average size of 159.8 by 38.2 μ. Others were fixed in warmed Fleming's fixative but there was no perceptible difference in the results produced by the two fixatives.

Epithelium.—With the exception of the apical papilla the outer surface of the body is covered by flattened, ciliated epidermal cells (Fig. 14). There are 20 of these cells arranged in 4 rows or bands which completely encircle the body. The anterior, or first, series consists of 6 cells, the second of 8, the third of 4, and the fourth of 2. Expressed by formula the arrangement is 6;8;4;2, in which the first number represents the most anterior row of cells.

The 6 cells of the anterior group cover the first fifth of the body, terminating posteriorly at the widest point in the body. The shape of the anterior part of the body is such that each cell of this group is wide at its posterior border and narrows gradually toward the anterior end, which lies at the base of the apical papilla. These cells are slightly thicker than the cells of the second group and consequently project above them very noticeably, and in slightly contracted specimens may overlap them for a

short distance. The thickness of the anterior cells is approximately 3 μ while that of the second group is 1.5 to 2 μ. The cilia present on the anterior group vary from 2 μ in length at the anterior tip of the cells to 12 μ at their posterior borders. The increase in length of the cilia is very gradual. Lynch (1933:15) states that the short cilia present at the anterior ends of these cells are stiff and motionless in the miracidium of *Heronimus chelydrae,* but they are movable in the present miracidium. As pointed out in the discussion on the hatching of the miracidium of *C. cotylophorum,* they are directed anteriorly during the hatching process but in free-swimming specimens they were observed to beat in the same manner as the other cilia.

The cells of the second group are rectangular and extend slightly past the middle of the body. The cells of the third group are also rectangular but are much broader, due to the fact that there are only 4 cells in this group and the body is only slightly smaller than in the region of the second group. The 2 cells in the posterior group cover the last fifth of the body and have a triangular shape because of the body form. Their broad ends are directed forward while the tapered ends cover the tapering posterior end of the body. All the cells of the 3 posterior groups are very uniform in their thickness, which is from 1.5 to 2 μ. The cilia also are very uniform in length, being approximately 12 μ in length on all of these cells. Each cilium is rooted in a distinct basal body. These bodies may be seen in living specimens as rows of very fine dots. Similar basal bodies have been described for the cilia of *Fasciola hepatica* by Ortmann (1908: 270; fig. 34a). The absence of cilia between the epithelial cells has been noted by most authors, but Talbot (1933:524; fig. 1) states that cilia cover the entire body of the miracidium of *Lechriorchis primus.* The spaces between the cells of the present miracidium are very narrow (Fig. 14), being from 1 to 2 μ, but the spaces between groups of cells are very distinct and can be seen especially well in optical sections of living miracidia. These spaces are most evident between the cells of the first and second groups because of the greater thickness of the first tier of cells. Because of this thickness the cilia on the first tier of cells project further from the body, and in swimming specimens they have the appearance of a mantle or epaulets and for this reason this region of the body is sometimes designated as the "shoulder" of the miracidium.

Ciliated plates or cells have been observed by other investigators on miracidia but the numbers of cells apparently are not the same even within a single species. A survey of the literature summed up in Table 7 shows the counts for different miracidia. Looss (1892: pl. 19, fig. 17) figured these cells on the miracidium of *Diplodiscus subclavatus.* He also figured them as being present on the miracidium of *Gastrothylax*

gregarius (1896: pl. 12, fig. 121) and *Gastrodiscus aegyptiacus* (1896: pl. 12, fig. 123) but did not give the number of cells present in these forms. However, in all of them he placed nuclei representing 4 tiers of cells, and it is very probable that the number and arrangement of cells

TABLE 7.—SHOWING THE ARRANGEMENT AND NUMBER OF THE CILIATED EPIDERMAL CELLS IN MIRACIDIA

Family	Genus and species	Author and date	Arrangement and number of cells	Total cells
Paramphistomidae	*Paramphistomum cervi*	Sinitsin 1931	6;6;3;4;2	21
	Cotylophoron cotylophorum	Bennett 1936	6;8;4;2	20
	Diplodiscus temperatus	Krull and Price 1932	6;8;4;2	20
Echinostomidae	*Hypoderaeum conoideum*	Mathias 1925	6;6;4;2	18
	Echinoparyphium recurvatum	Rasín 1933	6;6;4;2	18
	Echinostoma revolutum	Beaver 1936	6;6;4;2	18
Strigeidae	*Strigea tarda*	Mathias 1925	6;8;4;3	21
	Diplostomum flexicaudum	Van Haitsma 1931	6;8;4;3	21
Schistosomatidae	*Schistosomatium douthitti*	Price 1931	6;8;4;3	21
Fasciolidae	*Fasciola hepatica*	Thomas 1883	4-5;5-6;3;4;2	18–20
	Fasciola hepatica	Coe 1896	6;6;3;4;2	21
	Fasciola hepatica	Ortmann 1908	6;6;3;4;2	21
	Fasciola halli	Sinitsin 1931	6;6;3;4;2	21
	Fasciola californica	Sinitsin 1931	6;6;3;4;2	21
	Fascioloides magna	Sinitsin 1931	6;6;3;4;2	21
	Fasciolopsis buski	Barlow 1925	6;6;6;6;6	30
Troglotrematidae	*Paragonimus westermanni*	Ameel 1934	6;6-7;3;1	16-17
Heronimidae	*Heronimus chelydrae*	Lynch 1933	4-6;6-10; 3-6;1-2	16-22

in each miracidium is 6;8;4;2. Unfortunately, he did not figure the nuclei of these cells in the miracidium of *Paramphistomum cervi* (1896: pl. 12, fig. 125). Sinitsin (1931) has given the epidermal cells of the miracidium of *P. cervi* as 6;6;3;4;2, which is very different from the number of cells described as being present in other miracidia belonging to

the family Paramphistomidae. It is probable that Sinitsin was mistaken in the number of epithelial cells in this form, doubtless being influenced by the number of cells present in the other miracidia which he has apparently described correctly. We may then assume that the number of epidermal cells for the family Paramphistomidae may be expressed by the formula 6;8;4;2.

The formula for the epidermal cells of the Echinostomidae miracidia based on the same number and similar arrangement which has been described for the different species may be expressed as 6;6;4;2. On a similar basis the formula for the Strigeidae miracidia may be expressed as 6;8;4;2, and for the Schistosomatidae miracidia as 6;8;4;3. The Fasciolidae miracidia formula might be expressed as 6;6;3;4;2 were it not for the extremely aberrant number of cells described for the miracidium of *Fasciolopsis buski*. The cells of the Troglotrematidae and of the Heronimidae miracidia are not uniform in number for the single species described for each of these families.

Price (1931:703) has pointed out the possibility that the number and arrangement of the ciliated epidermal cells may be of some value in establishing relationships between families or larger groups. As she has said, the number of these cells possibly indicates relationship between the Strigeidae and the Schistosomatidae. Lynch (1933:10) has expressed doubt that these cells are of any value in determining relationships of various groups. However, the preponderance of the evidence points to the conclusion that the number and arrangement of these cells may be of value for establishing relationships within the families, and there is some evidence that it may be of value in establishing relationships between families. A final conclusion cannot be based on the small amount of information now available.

The nuclei of the epidermal cells were studied in living specimens stained with *intra vitam* stains, in stained toto mounts, and in stained sectioned material. The nuclei of the first group of cells are irregularly cylindrical in shape and are located near the posterior borders of the cells. The nucleus in each cell measures 7 to 10 μ by 2 to 3 μ in surface view. The shape of the nuclei of the second group of cells is so irregular that it may be described as being lobed. These nuclei also are located near the posterior borders of the cells and measure approximately 6 by 3 μ. The nuclei of the third group of cells are similar in appearance to those in the first group, although they are somewhat larger, being 9 to 12 μ by 2 to 3 μ in size. They are located very near the posterior boundaries of the cells. The nuclei in the last group of cells are found very near the anterior borders of the cells. These nuclei are larger than any of the others, measuring 12 by 3 μ, although their shape is much the same as

that of the nuclei in the first and third groups. The positions and shapes of the nuclei of all these cells as seen in cross sections are shown in Figs. 18, 19, 20, 21. The thickness of the nuclei as seen in cross section is about 1 μ.

The nuclei of the epidermal cells have been described for only a few miracidia. Thomas (1883: pl. 2, figs. 5, 6) figures them in the miracidium of *Fasciola hepatica* as round and located in the posterior part of the cells; Leuckart (1886:63; fig. 37) figures them as round and centrally located; Ortmann (1908: table 14; fig. 38) figures them as round in sectioned material and located toward the posterior border of the cells; Coe (1896:565) was the first author to describe in detail their shape and position. Sinitsin (1931:426; fig. 8) describes and figures these nuclei as being long and located at the posterior borders of the cells in the miracidia of *Fasciola halli, F. californica, Fascioloides magna,* and *Paramphistomum cervi.* Lynch (1933:20) saw them in the miracidium of *Heronimus chelydrae* as circular and flat in cross section. He could not see them in surface views and his statement as to their shape is open to question, since one might easily gain the impression that they are round when seen in sectioned material only. Krull and Price (1932:5; fig. 6) have described nuclei for the miracidium of *Diplodiscus temperatus* which closely resemble those of the miracidium of *C. cotylophorum.* The chief difference is that the nuclei of the second and third groups of cells are more anteriorly placed in the miracidium of *D. temperatus.*

Subepithelium.—The subepithelium is a thin, transparent layer located immediately beneath the ciliated epidermal cells and is continued forward at the anterior end to form the apical papilla (Fig. 17). The cell boundaries of this layer could not be determined but its extent is clearly indicated by the nuclei. The layer varies in thickness with the state of contraction of the miracidia, but in well extended specimens it is approximately 5 μ thick. It is somewhat less than this in the posterior region of the body, which is distended by the germ mass, and in the region of the body distended by the primitive gut and the brain. Slightly anterior to the middle of the body, between the brain and germinal tissue, there is an inward protrusion of this layer in which the flame cells are embedded. The nuclei are elongate or almost round and contain a number of small chromatin masses (Fig. 22) which aid in distinguishing these nuclei from the others of the body. The size of these nuclei is 4 to 6 μ by 3 to 4 μ. Krull and Price (1932:6; fig. 7) described and figured the nuclei of this layer as round and showed that they are arranged in 3 definite rows encircling the body of *Diplodiscus temperatus.* No other authors have described the nuclei of this layer as round or attempted to demonstrate that they are arranged in definite positions in the body.

In the present work a careful study of these nuclei was made and it was found that they vary from elongate to round in shape and that they are distributed in 4 principal groups (Fig. 15). However, these 4 groups do not contain all of the nuclei. There are many nuclei situated irregularly throughout the subepithelium, and a careful count of all the nuclei indicated that the number is far from constant. The first group, which is located beneath the anterior limits of the second group of ciliated cells, consists of from 10 to 20, with an average of 16. The second group is located in the middle of the body and the number of nuclei varies from 12 to 19, with an average of 15. The third group is located immediately beneath the termination of the third group of ciliated cells and variation in this group was found to be from 6 to 11, with an average of 8. The fourth group, consisting of from 2 to 4 nuclei, with an average number of 3, is located in the posterior extremity of the body. Some of these nuclei were occasionally seen undergoing mitosis, indicating still more the futility of attempting to determine the number of cells in the subepithelium. The presence of dividing nuclei in this layer indicates that the miracidium may grow while free-living but no experiments were made to determine the correctness of this supposition.

Muscle Tissue.—The rapid and strong contractions and extensions of the miracidium indicate a well developed musculature. The muscles observed in this miracidium were the circular and longitudinal layers which are between the ciliated epidermal plates and the subepithelium. The layer of circular muscles is located external to the longitudinal layer and the two are pressed closely together (Fig. 23). The circular muscles can be seen in living specimens and sectioned material as minute parallel bands closely set together. A single band or fiber measures approximately 1 μ in diameter and the distance between fibers is about 1 μ.

The longitudinal muscles are slightly more developed than the circular muscles in the anterior region of the body. These muscles are arranged in parallel bands also. Each band measures approximately 1.5 μ in diameter, and the distance between bands is about equal to their diameter. The greater development of the fibers at the anterior end of the body is due, perhaps, to the fact that they serve to retract and extend the apical papilla and the anterior region of the body.

The muscles in the posterior regions of the body are extremely difficult to demonstrate but their presence is indicated by the ability of the miracidium to extend and contract this region. The circular muscles can be seen in living and stained toto mounts and the arrangement is the same as in the anterior region of the body. The longitudinal muscles were never seen clearly in this region of the body.

The arrangement of muscles in miracidia has been described by several

authors and there is close agreement between their descriptions. Ortmann (1908) describes and figures the muscles of the miracidium of *Fasciola hepatica* as having an arrangement very similar to that of *C. cotylophorum*. Looss (1892, 1896) describes and figures the muscles of the miracidia previously mentioned as being the same as in the closely related miracidium described here. Reisinger (1923:12; fig. 3) describes and figures the muscles of the miracidium of *Schistosoma haematobium*. He describes the circular muscles as bands 0.6 by 0.1 μ placed at intervals of 0.8 to 1.1 μ. The longitudinal muscles are similarly arranged but measure only 0.5 μ in breadth and are placed at intervals of 2 to 4 μ. The muscles of this miracidium are much smaller than those of *C. cotylophorum* but the two agree as to arrangement. The retractor muscles described by Reisinger as being present at the anterior end of the body could not be seen. Ishii (1934:30) describes large muscle cells containing nuclei in the miracidium of *Fasciolopsis buski* but similar structures were not seen in the present material.

Primitive Gut.—The structure usually called the primitive gut by writers in their descriptions of miracidia is a flask-shaped structure of variable proportions, depending on the state of contraction of the miracidium. In elongated specimens it may extend slightly posterior to the middle of the body, while in contracted specimens it becomes broadened and occupies all of the available space in the anterior fourth of the body. In specimens of average extension it extends almost to the center of the body. It terminates anteriorly in a narrow duct which does not open to the outside. The coarsely granular contents first appear when the gut consists of only two cells (Fig. 9). Later the two original cells divide and the four resulting cells apparently become confluent, because no cell boundaries can be found. The nuclei remain in the posterior part at all times and are surrounded by cytoplasm which stains more darkly than any other region of the gut. The granular contents move freely and completely fill the gut with the exception of the anterior tip of the duct near its termination in the apical papilla. The nuclei are easily recognized in all preparations of the miracidia because of their position and size. They measure 5 to 7 μ in diameter and contain several small masses of chromatin, usually grouped together near the center of the nucleus.

The development of the primitive gut at some distance from the anterior end of the body, the size of the cells and their nuclei, the early development of the granular contents, the absence of a definite cell wall around each nucleus after the four-cell stage is reached, the concentration of cytoplasm around the nuclei at the posterior end of the gut, the absence of a mouth and a lumen, and the complete disappearance of the contents immediately after penetration of the miracidium into the snail

host while the nuclei may still be identified—all give evidence in favor of interpreting this structure as being a gland rather than a gut.

The earlier writers—Schauinsland (1883), Thomas (1883), Leuckart (1886), Looss (1892, 1896), Coe (1896), and Ortmann (1908) as well as some of the later writers—Faust and Meleney (1924), Mathias (1925), Barlow (1925), Sinitsin (1931), and Van Haitsma (1931)—considered this structure as a primitive or vestigial gut. More recently, Reisinger (1923), Manter (1926), Price (1931), and Lynch (1933) have presented evidence in favor of considering this structure as a gland. An analysis of the opinions of these writers leaves some doubt as to the nature of this organ, but I believe, for the above reasons, that it is a gland and that it functions during the penetration of the miracidium into the snail.

Penetration Glands.—These glands have been described for many miracidia, but within the family Paramphistomidae they have been observed only in the miracidium of *Diplodiscus temperatus* by Krull and Price (1932:7; fig. 7). Looss (1892, 1896) does not describe them for the miracidia of the forms previously mentioned, all of which belong to this family. Krull and Price found two pairs of these glands, a pair being situated on each side of the gut. In *C. cotylophorum* there are two pairs of these glands which are extremely hard to detect. However, they may be observed late in the development of the miracidium as well as in the mature specimens (Figs. 11, 17). They are filled with a clear nongranular substance which is difficult to stain. They extend posteriorly for about one-fifth of the body length from their openings at the base of the apical papilla. The nuclei of these cells, which are located near their posterior ends, measure 4 to 5 μ in diameter.

Nervous System and Sense Organs.—The nervous system consists of a central fibrous mass, nerves, and nerve cells. The central fibrous mass is located dorsal to the posterior part of the primitive gut, where it may be seen easily in both living and stained specimens surrounded by the nuclei of the nerve cells (Fig. 22). In mounted specimens the brain appears to be lateral in position, due to the pressure of the cover slip (Fig. 17). It is oval or quadrangular in shape when viewed from the dorsal side and measures approximately 20 by 25 μ. It is from 14 to 16 μ in depth and characteristically forms an indentation in the dorsal side of the primitive gut, which can be seen in lateral view.

No nerves passing out from the brain could be seen in living specimens stained *intra vitam* or in stained toto mounts. It was in sectioned material only that large fibrous structures resembling nerves were found arising principally from the lateral surfaces of the brain, although smaller fibers were found arising at various other points. Two large

fibers were found passing obliquely forward from the brain to the lateral processes located between the first and second rows of ciliated epidermal cells (Fig. 22). While similar processes or nerves were observed to leave the brain from its posterior lateral surfaces, they could not be traced to their terminations. Neither could the smaller nerves be traced to their terminations, although finer striations observed in longitudinal sections of the anterior part of the body may have been nerves passing to terminations in this region.

The nerve cell boundaries were not seen at any time but their nuclei are stained readily by *intra vitam* stains and by other stains used on sectioned material. These nuclei are located in largest numbers immediately anterior to the brain mass, although a few were found scattered completely around it. These nuclei were found to vary from 12 to 20 in number while the average number was 17. No nerve connections could be traced to or from them. In appearance they greatly resemble subepithelial nuclei but they are round and slightly smaller, and the dense chromatin causes them to stain more darkly. They vary in size from 2.6 to 3.4 μ in diameter.

Looss (1896: pl. 12, figs. 119, 121, 125) has figured a central fibrous nerve mass surrounded by nuclei for the miracidia of *Gastrothylax gregarius, Gastrodiscus aegyptiacus,* and *Paramphistomum cervi* which is very similar to that of the miracidium of *C. cotylophorum.* He also observed anterior and posterior nerves arising from each side of the fibrous nerve mass in the miracidium of *Gastrothylax gregarius.* Krull and Price (1932) described a central mass for *Diplodiscus temperatus* but did not find the anterior and posterior nerves. However, they did describe 6 nerve cells located anterior to the brain which had fibers connecting the brain and the tip of the apical papilla. These cells were not observed in the present species. Reisinger (1923:16; fig. 3) in his description of the nervous system of the miracidium of *Schistosoma haematobium* found structures similar to those found in the present material, as did Lynch (1933:22; fig. 9) in the miracidium of *Heronimus chelydrae.*

The only sense organs observed on the miracidium of *C. cotylophorum* were a pair of structures which have been variously named anterior ducts, anterior papillae, mucoid secretion, lateral papillae, and lateral processes by different authors. These organs are located laterally between the first and second rows of ciliated epidermal cells (Fig. 14). Each papilla appears as a clear structure, approximately hemispherical in shape. It was difficult to make out any internal structures connected with these papillae but at times in living miracidia there appeared to be a duct extending inwardly and posteriorly from each papilla which seemed to terminate in a small vesicle in the region of the central nerve mass.

This structure is probably the large nerve previously described as passing from the brain to the lateral papilla.

Cort (1919:516) and Faust and Meleney (1924:28) called these papillae anterior or lateral ducts and stated that the extrusion of substances from these structures was observed. Stunkard (1923:183) made similar observations on the miracidia of Spirorchis. The present observations agree with those of Sewell (1922:287) who found no openings in these structures in the miracidia of *Cercariae Indicae* xv. Price (1931: 705) made similar observations on the miracidia of *Schistosomatium douthitti.* Living miracidia of *C. cotylophorum* were observed for great lengths of time and even when subjected to pressures great enough to break the body walls no substance was observed to be extruded.

Looss (1896: fig. 125) figures but does not discuss two small lateral papillae on each side of the miracidium of *Paramphistomum cervi.* Krull and Price (1932: fig. 7) figure one lateral papilla on each side of the miracidium of *Diplodiscus temperatus* but they show no structures in connection with them nor do they suggest the possible function of these papillae.

That these structures are sensory in *Schistosoma haematobium* was clearly demonstrated by Reisinger (1923) who has described and figured nerves passing from the central nerve mass to them. Lynch (1933:31; fig. 9) has demonstrated in a similar way that these papillae are sensory in the miracidium of *Heronimus chelydrae.* The present observations agree in detail with those of these two workers and I believe that these structures are sensory.

Many other structures designated as sensory in function have been described for other species of miracidia by Coe (1896), Ortmann (1908), Price (1931), Reisinger (1923), and Lynch (1931) but none of these were found in the present material.

Germinal Tissue.—The germinal tissue was studied from its first appearance in the developing embryo and the conclusions arrived at are a result of detailed study on living material, stained toto mounts, and serial sections. In the youngest forms in which the germinal tissue could be distinguished it had a homogeneous translucent appearance. Nuclei 4 to 5 μ in diameter were present. As the miracidium develops some of the germ cells break loose into the central cavity. One large and several small germ balls usually are present when the miracidium is hatched, although some are hatched in which no definite germ balls have developed. In the latter cases there are many germ cells which seem to be free in the central cavity. The posterior three-fifths of the body is completely filled by the germinal tissue in the fully developed miracidium.

There is a pronounced difference in the appearance of this tissue and

the enclosing subepithelium in stained specimens. The nuclei of the subepithelium of this region are ovoid and do not stain as darkly as do the round nuclei of the germinal tissue. No cell boundaries could be distinguished but the limits of the germinal tissue could be established by the numerous large, closely packed nuclei, the granular appearance of the tissue, and its much greater affinity for stains than the subepithelial layer. The germinal nuclei, representing the germ cells, form a thick layer in the posterior extremity of the body and are located also around a central cavity which extends forward past the middle of the body (Fig. 16). These nuclei, which measure 4 to 5 μ in diameter, have numerous small scattered chromatin masses and one large centrally located mass. The amount of cytoplasm surrounding each of these nuclei is very small. After being liberated into the central cavity the germ cells develop into germ balls by unequal cleavage (Fig. 17). Early in cleavage a thin membrane encloses the developing germ ball. This membrane is present around each of the germ balls and it seems to develop from very small cells located in the periphery of the germ balls. Ortmann (1908:287) discusses a similar structure in the miracidium of *Fasciola hepatica* and Lynch (1933:29) has done the same for the miracidium of *Heronimus chelydrae*. The observations made by Looss (1892), Price (1931), and Lynch (1933) that the germ balls are held in position by fiber-like attachments were not confirmed in the present material. Germ cells located in the posterior part of the body at the end of the central cavity were observed to be attached to the lateral germinal areas by extensions of the cells but no connections were found for the cells which were free in the cavity or for the germ balls.

Excretory System.—The excretory system is very similar to that described in miracidia of many species. It consists of two laterally situated flame cells and their ducts (Fig. 15). The flame cells are located in the previously mentioned protrusion of the subepithelial layer, immediately anterior to the middle of the body. Each flame cell measures approximately 10 by 3 μ. Reisinger (1923) in his detailed study of the excretory system of the miracidium of *Schistosoma haematobium* states that a flame cell nucleus is lacking. There are several nuclei embedded in the tissue surrounding the flame cells of the present material, but since cell boundaries were not seen no nucleus could be definitely associated with the flame cell. However, a nucleus was found in close proximity to the basal plate of the cell (Fig. 13) which is probably directly associated with it. The excretory tubule for each flame cell extends posteriorly in loose coils almost to the excretory pore. Here it loops back to the flame cell where it again turns on itself and extends to the excretory pore which is located laterally between the third and fourth epidermal plates.

Immediately before reaching the pore the tube is expanded to form a small excretory bladder. Krull and Price (1932:8; fig. 8) describe two duct nuclei for each of the tubules in the miracidium of *D. temperatus,* but these nuclei could not be found in the miracidium of *C. cotylophorum.*

INTERMEDIATE HOST

Determination of the Host

Two methods were used in determining what snail or snails would serve as the intermediate host of *C. cotylophorum.* The first consisted of searching known foci of infestation for infested snails, and the second consisted of exposing several species of snails to the free-swimming miracidia followed by dissection of the snails after an interval of 10 or 15 days to discover whether or not there were any developmental stages present in them.

Natural Infestation.—The distribution of naturally infested snails depends entirely upon the distribution of infested carriers of the adult worm. Consequently, in order to determine what region of the country surrounding Baton Rouge, Louisiana, had the largest number of carriers, many of the cattle slaughtered at the city *abattoir* during the period from June 6, 1933, to June 27, 1934, were examined for these worms. When an infested cow was found, the range from which it came was located and searched for the intermediate host of *C. cotylophorum.* It soon became evident that the worms were more abundant and more frequently found in cows which came from the low, semi-swampy ranges located south and east of the city. Occasionally cows from the hilly ranges north of the city were found to be infested and upon examination of the ranges from which these came it was found that they had access to either a small pond or lake or to a stream which ran through open fields. It was not until May 26, 1934, that a natural infestation was found in specimens of *Fossaria parva* taken from the margin of an artificial pond southeast of Baton Rouge.

Experimental Infestation.—The presence of strongly developed cilia combined with the swimming ability of the miracidium gave basis for a surmisal that the intermediate host was either an aquatic or amphibious snail. Several genera of snails are commonly present in or around streams, lakes, and ponds in this region. Prior to the finding of naturally infested intermediate hosts, snails were collected from an area semicircular in shape with a radius of approximately 20 miles, being taken in every instance from a range on which the cattle were known to be infested with *C. cotylophorum.* The snails were examined first by placing

them in water to determine whether or not fully developed cercariae were present, and then by dissection for the earlier developmental stages. When found not to be infested others were then exposed to free-swimming miracidia. The snails used in these experiments were *Physa halei, Helisoma lentum, Succinea retusa, Succinea unicolor, Fossaria parva, Fossaria modicella,* and undetermined species of Physa and Campeloma. Of these only *Fossaria parva* and *F. modicella* could be infested experimentally.

F. modicella was found in only one locality near Baton Rouge, Louisiana, late in the spring of 1934, and consequently is not used in the following discussion on the development of larval stages of *C. cotylophorum.* Snails of this species collected from near Urbana, Illinois, by the writer and from Turkey Run State Park, Indiana, by Dr. H. J. Van Cleave were infested experimentally also. Krull (1934:171) secured this same species from Utah and was able to infest these snails with the miracidium of *C. cotylophorum. F. parva* was found frequently and doubtless is the species which serves most often as the intermediate host of this parasite in the vicinity of Baton Rouge.

Biology of *Fossaria parva*

Fossaria parva is a small amphibious snail which rarely exceeds 7 mm in length. It is commonly found near the margins of small ponds, lakes, and streams where there is some decaying vegetation which it uses for food. The snails were rarely found in the water, and they are very helpless when caught by any current, being carried along until they drift against some object. They were never found in areas which were shaded at all times. The normal habitat is a well moistened area which is subject to direct sunlight for the greater part of the day.

The snails were found in largest numbers along the margins of an artificial pond which served as a source of drinking water for cattle, and along the margins of a large unshaded drainage ditch. Observations were made at intervals on the snails present in the drainage ditch. They were first located in August, 1933, and were present in large numbers on the moist area at the edge of the water. As the water receded during dry periods it was followed by the snails and when the ditch became completely dry the snails burrowed into the mud where they remained until water was again present. Snails were collected in large numbers from this ditch during the autumn and early winter months but none could be found after December 26, 1933. Observations were continued through January and February, 1934, but no snails were seen before March 3. At this time a single specimen was found after an hour of

searching. One week later 4 specimens were collected. These specimens were all large. By the latter part of March these older specimens were found frequently and very young snails were observed in steadily increasing numbers during the month of April.

The first snails used for experimental purposes were collected during September, 1933, from the previously mentioned drainage ditch which was not accessible to carriers of the adult worms. However, many of the snails were examined for developmental stages but not a single naturally infested snail was found throughout the months in which collections were made from this locality. These snails were placed in aquaria which were prepared to approximate as nearly as possible the natural habitat of the snails. Large and small galvanized tin tubs were filled with dirt taken from the natural habitat and this dirt was banked to one side so that when water was added there was a dry area at the top of the bank, a moist area along the water's edge, and then water which was kept at a fairly constant level. To make the habitat still more natural, grasses, weeds and aquatic vegetation were planted in the aquaria. These aquaria were placed against the southern wall of a large building where they were exposed to the sunlight for the greater part of each day. They remained uncovered except during rains. To supplement the food supply planted in the aquaria, lettuce, cabbage and cauliflower leaves were added when needed. None of the vegetation available to the snails was eaten until it was partially decayed. The snails kept under these conditions seemed to live as well as in their natural habitat. The rate of reproduction was rapid and a constant supply of laboratory raised snails was available after the first few weeks. Under these conditions the snails did not hibernate during the colder months, i.e., January and February, and there was some reproduction although less than in the warmer months. The eggs are usually deposited at the edge of the water but are sometimes found in moist depressions some distance from the water.

The snails were very seldom found in the water or at the dry top of the bank, preferring in the aquaria the same habitat as in nature. Usually when found in water they are attached to some piece of decaying vegetation.

Penetration Experiments.—The snails were infested by placing them singly in small glass dishes containing from 3 to 5 miracidia or by exposing a large number of snails to hundreds of free-swimming miracidia in large containers. The miracidia are attracted to the snails almost immediately, but apparently penetration takes place slowly and only after prolonged exposure of the snails to them. Observations were made many times on the reactions of the miracidia but none was ever observed to

penetrate the snail. In experiments with only a few miracidia in a dish with a single snail they have been observed for as long as 3 hours and at the end of this time all of the miracidia were still present in the dish. Possibly the constant activity of the snail during observation under the microscope and the abnormal conditions under which the miracidia are placed prevented them from entering the snail. The percentages of infestation were always low when the snails were exposed to only a few miracidia, usually about 10% becoming infested. The method of exposing snails to many miracidia resulted in 100% infestation in most cases, but there is danger of over-infestation. However, it was found that the best results could be obtained by exposing the snails in this way for a period of 2 to 4 hours. In earlier experiments snails were left overnight in dishes containing many miracidia, but the subsequent death rate among the snails was so great that only an occasional individual would live long enough to shed cercariae.

The natural inclination of the snails to leave the water adds to the difficulties in securing 100% infestation. To secure the best results in this respect it was found necessary to place in the containers some food material which served to attract the snails into the water. Under these conditions the snails remain relatively motionless, giving the miracidia ample time and opportunity to penetrate them. The miracidia collect at the anterior ends of the snails where they can be seen attaching themselves to the head, foot, and mantle, and at times to the shell. Their attachment usually is very brief, as they are shaken off by slight movements of the snail or they release themselves. They may then attach themselves again at the same point, select another, or swim away, finally becoming attached to another host. At times the snails seem to have no attraction at all for the miracidia. In such cases miracidia were observed swimming in close proximity to several snails but never made any attempt to penetrate them. In view of some of the recent observations on immunity of infested snails this failure of miracidia to penetrate might be due to earlier infestation by this same or other trematode larval forms, but extended observations failed to yield any evidence of natural infestation in the stock of snails used in these experiments. Experiments also demonstrated that these snails do not become immune to infestation by the miracidia of *C. cotylophorum* when previously infested by this same species. Snails containing larval stages as much advanced as mature rediae were exposed to miracidia, and large numbers of the latter penetrated and began development. Miracidia were observed collecting around snail faeces and débris scraped from the bodies of the snails. These substances produced reactions in the miracidia alike in all respects to those produced by the snails themselves. Apparently the attraction was as

strong as that of the snails since the miracidia continued to collect around them even though there were many snails in the container.

The penetration of the miracidia into the snail host was never observed, even though many hours were spent in attempting to do so. However, Krull (1934:176) observed that this miracidium penetrated the head and mantle of *Fossaria modicella* within 15 minutes. In order to determine the point of entrance in the present experiments a few medium-sized snails were exposed to large numbers of miracidia for 6 hours and were then fixed. Upon sectioning and staining these snails many miracidia were found to have penetrated all the exposed surfaces of the snail but principally the mantle and dorsal surface of the foot.

The habitat of *F. parva* and the fact that it feeds on moist decaying vegetation at the edge of the water, where eggs deposited in the faeces of cattle would remain viable, led to experiments to determine whether or not ingested eggs would hatch in the intestine and the miracidia penetrate the intestinal wall. Some small snails were placed in a dish containing eggs almost ready to hatch which were readily eaten by the snails. A few of the snails were fixed immediately after eating the eggs, while the rest were kept for 24 hours before being fixed. It was noticed that some of the eggs were passed in the faeces of the snails unhatched and unharmed since many of them were observed to hatch subsequently. The fixed snails were sectioned and stained, and a careful search was made for miracidia which might have penetrated the wall of the digestive tract at any point, but none was found.

Numerous experiments were made during the fall and winter months of 1933 with very young, medium-sized, and old snails and it was found that the miracidia infested all ages equally well. However, in these experiments large numbers of miracidia were used and as a result most of the snails died before cercariae were shed. The young snails when severely infested usually die within 15 to 20 days while the older ones live until the liver is completely destroyed by developing cercariae. In the young snails which died only sporocysts and young rediae were found.

Relation of Temperature to Development.—One group of snails infested on October 5, 1933, shed cercariae on November 17, after a lapse of 44 days. A second group infested on October 31 shed cercariae on December 23, after 54 days, and a third group infested on December 20 shed cercariae on March 30, 1934, after 91 days. The time required for development increased as the winter months advanced. Late in the spring and in the early summer months of 1934 another series of experiments was performed and in this series the time required for development decreased as the temperature rose. Snails infested on April 25

shed cercariae on June 1 (37 days); a second group infested on April 26 shed cercariae on June 2 (37 days); a third group infested on May 5 shed cercariae on June 5 (31 days); a fourth group infested on May 11 shed cercariae on June 12 (32 days); a fifth group infested on May 15 shed cercariae on June 14 (30 days); and a sixth group infested on June 23 shed cercariae on July 24 (31 days). The results of these experiments are summarized in Table 8.

TABLE 8.—DATA SHOWING TIME REQUIRED FOR DEVELOPMENT OF CERCARIAE OF *Cotylophoron cotylophorum* AT DIFFERENT TIMES OF THE YEAR

No.	Date of infestation	Date on which cercariae were shed	Days elapsed
1	Oct. 5, 1933	Nov. 17, 1933	44
2	Oct. 31, 1933	Dec. 23, 1933	54
3	Dec. 20, 1933	Mar. 30, 1934	91
4	Apr. 25, 1934	June 1, 1934	37
5	Apr. 26, 1934	June 2, 1934	37
6	May 5, 1934	June 5, 1934	32
7	May 11, 1934	June 12, 1934	32
8	May 15, 1934	June 14, 1934	30
9	June 23, 1934	July 24, 1934	31

These data show that the effect of temperature on the rate of development of the stages in the snail host is very comparable to the effect on the rate of development of the miracidium. The time required for the development of the miracidium increased from 11 days in the warmer months to 29 days during the colder months of the year. In the same way the time required for the development of the cercariae increased from 30 to 91 days. Krull (1934:174) found that snails infested with the miracidium of *C. cotylophorum* on May 14 began to shed cercariae on June 19, after a lapse of 36 days. This result agrees very closely with the foregoing since snails infested on May 15 began to shed cercariae on June 14, representing a difference of only 6 days from Krull's data. Krull performed only the one experiment so that no other comparisons can be made. Suzuki (1931:97) performed similar experiments on the development of *Fasciola hepatica* and obtained results comparable to those presented here. He found that a period of 30 days was required for the development of these stages during the summer months of July and August but that from 60 to 70 days were required during the winter months of January and February.

The data secured on the time required for the development of the cercariae during the warmer months indicate that 30 days is approximately the minimum time required. The average time required for de-

velopment of the cercariae during these warmer months was 33 days. The rate of development of the sporocyst, redia, and cercaria was studied in all the experiments shown in Table 8—by dissection of snails at intervals, and also by fixing, sectioning, and staining snails. In this way it was possible to determine at what time the various developmental stages make their appearance. The following discussion is based on the development of these stages in snails infested on May 5. Cercariae were shed by these snails on June 5, after a period of 32 days.

SPOROCYST

Development

Method of Study.—The earlier stages in development of the sporocyst were studied from sectioned material only. The minuteness of these stages combined with the opacity of the snail shell made accurate observations of living material impossible. To obtain representative stages some of the snails were fixed at 12 hours, and others after 1, 2, 5, 10, 15, 20, 25, 30, and 35 days from the time of exposure to miracidia. In order to supplement the data acquired from sectioned material many snails were dissected after the fifth day of development, at the ends of the given periods and at intervals not indicated, in order to study the living forms.

Sporocyst from Penetration of Miracidium to End of 12 Hours.—As has been stated previously, the miracidia were not observed to penetrate the snail host, but it was possible to determine the points of entrance from sectioned material. The miracidia were never observed to shed the ciliated epidermal cells and evidence from sectioned individuals within the snails clearly demonstrated that the epidermal cells are present after penetration. In several instances miracidia were found in the lymph spaces of the foot and in the body cavity, with all or a part of the cells still attached (Figs. 24, 29). The exact time at which they are lost was not determined but there is no evidence of their presence 12 hours after penetration. The cells are sloughed first from the anterior end of the body as indicated by the fact that many individuals were found on which only the last one or two tiers were present. The opposite condition was never observed. Looss (1892:156) observed that the ciliated cells of the miracidium of *Diplodiscus subclavatus* were retained for 24 hours and until it had reached the liver or ovo-testis. The miracidium of *P. cervi* according to Looss' description (1896:186) does not lose its ciliated cells until after penetration and changes accompanying transformation

into the sporocyst have begun. Takahashi (1928:278) made similar observations on the miracidium of *P. cervi.* Thomas (1883:114; Fig. 7) observed the same phenomenon in the miracidium of *Fasciola hepatica.* He states:

> The outer layer of ciliated cells is lost, whilst the embryo changes in form. The ciliated cells absorb water and appear as round or hemispherical vesicles with the cilia standing out perpendicularly from their surface . . . .

He does not state how soon after penetration these cells are lost, and it was not determined for the present miracidium. However, they were seen only on miracidia which had undergone the least change at the end of 12 hours after penetration. Doubtless these changes are initiated immediately after the miracidium gains entrance into the snail.

Ameel (1934:289) observed the penetration of the miracidium of *Paragonimus westermanni* but could not determine whether or not these cells are lost although Nakagawa (1917:302) noted that the cells are lost during penetration. Mathias (1925:44; fig. 9a) has described and figured the loss of these cells during the penetration of the miracidium of *Strigea tarda.* Barlow (1925:34; text-fig. 6) made similar observations on the miracidium of *Fasciolopsis buski* as did Ishii (1934: figs. 1, 2, 3) for the same form. Rasín (1933:102) observed that the epidermal cells are shed by the miracidium of *Echinoparyphium recurvatum* before entrance into the snail host. In view of these observations it is only possible to conclude that the ciliated epidermal cells are lost by some miracidia during penetration but are carried into the snail host by others.

The appearance of the miracidium is not greatly altered for some time after penetration, many of the structures being still recognizable. The structures most altered in appearance are the primitive gut and the brain. No contents can be seen in the gut even in miracidia which have just penetrated the snail, as indicated by their very superficial positions. The nuclei of the gut are recognizable in some miracidia but they disappear before the end of the first 12 hours after penetration. The brain degenerates rapidly, not being definitely recognizable in any miracidia after penetration. In a number of miracidia a small round structure was observed, occupying the position of the brain in the free-swimming forms, which might have been the brain in an advanced state of degeneration. This structure measured 12 μ in diameter.

The nuclei of the penetration glands were not seen in miracidia after penetration, but occasionally individuals were seen in which the ducts of the glands were very conspicuous. In some individuals at the end of the first 12 hours the anterior part of the body possesses no recognizable structures other than a few nuclei (Fig. 29).

The appearance of the subepithelial cell nuclei remains the same

through the first stages of transformation but there is a great reduction in number. The central cavity of the miracidium remains and one or two germ balls and many germ cells are present in it. The excretory system is unchanged from that typical of the miracidium.

As transformation takes place the miracidium loses its elongate shape and becomes gradually more ovoid. A very thin cuticula is formed around the outside of the body. Accompanying the change in shape and the loss of miracidial organs is a decided decrease in size. The size of 10 sporocysts which were approximately 12 hours of age is given in Table 9.

Table 9.—Showing the Size (in Millimeters) of 12-Hour and 24-Hour Sporocysts

No.	12 Hours	No.	24 Hours
1	0.070 x 0.035	1	0.077 x 0.024
2	0.054 x 0.032	2	0.069 x 0.038
3	0.052 x 0.037	3	0.069 x 0.033
4	0.052 x 0.025	4	0.069 x 0.024
5	0.048 x 0.032	5	0.064 x 0.030
6	0.046 x 0.031	6	0.061 x 0.032
7	0.046 x 0.024	7	0.061 x 0.030
8	0.045 x 0.034	8	0.061 x 0.026
9	0.045 x 0.029	9	0.061 x 0.023
10	0.039 x 0.032	10	0.060 x 0.018
Average	0.050 x 0.031	*Average*	0.065 x 0.028

Barlow (1925:34) observed that the sporocysts of *Fasciolopsis buski* never became immobile and that the digestive tract became larger and functionally more active as the sporocysts grew in size and that they could be observed feeding at all times. Before rediae were born he found that the sporocysts had migrated well into the body of the snail. These observations could not be confirmed in the present material. The positions in which the sporocysts develop indicate that some of the miracidia either swim in the body fluids or are passively carried by movements of the liquids in the cavities of the snail's body. However, they become permanently located soon after penetration. In some instances young sporocysts were found free in the body spaces surrounding the radula and esophagus, and subsequent findings indicate that sporocysts develop attached to the walls of this cavity. Other young sporocysts were observed in all parts of the foot, including the center of this muscle mass, which further demonstrates that some movement from the point of penetration does occur. The sporocysts apparently prefer the mantle tissues to any other tissues of the snail's body since more of them develop in this region than elsewhere in the body.

24-Hour Sporocyst.—The initial rate of development is very slow when the size of the sporocyst is taken as a criterion. Perhaps this is due to the fact that an almost complete transformation occurs and to the fact that the sporocyst must become established before it receives adequate nourishment to provide for rapid growth. There are two notable changes which occur by the end of the first 24 hours in the snail. The first is the initiation of growth, as shown in Table 9, and the second is the breaking down of the germ balls which were present in the miracidium (Fig. 28). The germ cells, separated from each other by the breaking down of the germ balls, are scattered in the central cavity, almost occluding it. No cell boundaries could be distinguished, thus giving the body the appearance of a syncytium. The germ cell nuclei measure from 4 to 6 μ in diameter and have lost the appearance characteristic of these nuclei in the miracidium. The chromatin is no longer concentrated in one central body surrounded by distinct masses but is uniformly scattered throughout the nucleus. Suzuki (1931: figs. 12, 13) has figured a similar stage in the young sporocyst of *Fasciola hepatica.* Mathias' (1925:44) description of this stage of development of the sporocyst of *Strigea tarda* is not complete but apparently it is very similar to that of the present material. Brooks (1930:302; fig. 1) has described and figured these scattered germ cells in the young sporocyst of *Cercaria lintoni* Miller which he designates as "antecedent germ cells."

Several elongate nuclei which measure 4 by 2 μ were observed near the periphery of the body. These are probably subepithelial nuclei. The anterior end of the body at this age appears as a translucent, granular mass in which remains of miracidial structures can be seen occasionally.

48-Hour Sporocyst.—After two days in the snail host the sporocyst no longer has any trace of the miracidial structures, although the anterior end of the body is still filled with a translucent tissue in which only an occasional nucleus is located. There is a decided increase in size over the 24-hour stage (Table 9). The most important development is that of the embryonic rediae. The origin of these was not established but it is possible that they are derived from the germ cells liberated by the breaking down of the miracidial germ balls, the "antecedent germ cells" of Brooks. The "germ mass" and "components" described by Brooks as being derived from the "antecedent germ cells" in the sporocyst of *C. lintoni* were not observed in the sporocyst of *C. cotylophorum.* The fact that the structures seen in the 48-hour sporocyst are embryonic rediae and not "germ masses" is clearly demonstrated by their subsequent development.

The size and number of young rediae present in 48-hour sporocysts is shown in Table 10. At a very early age these embryos have a definite

TABLE 10.—SHOWING THE SIZE (IN MILLIMETERS) OF 48-HOUR, AND 5-, 10-, AND 15-DAY SPOROCYSTS, THE NUMBER OF REDIAE IN EACH, AND THE SIZE OF THE LARGEST REDIA IN EACH

Age and No.	Sporocyst	Number of rediae	Largest redia
48 hours:			
1	0.070 x 0.038	1	0.023 x 0.016
2	0.070 x 0.053	1	0.015 x 0.015
3	0.077 x 0.033	1	0.023 x 0.015
4	0.082 x 0.043	2	0.025 x 0.018
5	0.092 x 0.046	1	0.018 x 0.018
6	0.100 x 0.046	3	0.021 x 0.015
7	0.101 x 0.046	2	0.023 x 0.018
8	0.107 x 0.053	3	0.021 x 0.020
9	0.123 x 0.026	2	0.023 x 0.015
10	0.123 x 0.028	2	0.027 x 0.018
Average	0.095 x 0.041	..	0.022 x 0.017
5 days:			
1	0.126 x 0.084	3	0.042 x 0.042
2	0.138 x 0.061	3	0.053 x 0.043
3	0.161 x 0.053	3	0.046 x 0.046
4	0.168 x 0.097	4	0.063 x 0.050
5	0.168 x 0.105	5	0.067 x 0.054
6	0.170 x 0.078	4	0.052 x 0.052
7	0.170 x 0.110	5	0.069 x 0.053
8	0.200 x 0.086	4	0.058 x 0.058
9	0.226 x 0.084	5	0.073 x 0.047
10	0.218 x 0.092	5	0.080 x 0.063
Average	0.181 x 0.085	..	0.060 x 0.051
10 days:			
1	0.160 x 0.080	5	0.076 x 0.029
2	0.218 x 0.063	5	0.105 x 0.038
3	0.260 x 0.134	5	0.105 x 0.055
4	0.268 x 0.151	5	0.139 x 0.092
5	0.294 x 0.105	8	0.151 x 0.046
6	0.302 x 0.100	5	0.134 x 0.088
7	0.336 x 0.134	5	0.126 x 0.084
8	0.395 x 0.189	5	0.168 x 0.050
9	0.420 x 0.168	7	0.189 x 0.088
10	0.433 x 0.160	9	0.189 x 0.046
Average	0.309 x 0.126	..	0.138 x 0.062
15 days:			
1	0.273 x 0.189	5	0.168 x 0.050
2	0.294 x 0.160	6	0.197 x 0.055
3	0.315 x 0.156	5	0.216 x 0.061
4	0.315 x 0.210	6	0.193 x 0.046
5	0.320 x 0.210	5	0.155 x 0.080
6	0.323 x 0.151	7	0.210 x 0.050
7	0.370 x 0.181	8	0.168 x 0.063
8	0.376 x 0.134	8	0.189 x 0.055
9	0.420 x 0.189	9	0.189 x 0.063
10	0.470 x 0.285	8	0.225 x 0.080
Average	0.348 x 0.186	..	0.191 x 0.060

shape and each is enclosed in a firm membrane which Dubois (1928:63) designates as a primitive epithelium (Fig. 31). In addition to the definitely formed rediae there are many germ cells present in the posterior part of the body. These cells measure 8 to 10 μ in diameter and have large nuclei which measure 6 to 7 μ in diameter. The cytoplasm of these cells stains more darkly than the surrounding tissues in which cell boundaries are not distinguishable. The chromatin of the nuclei is arranged in one large body eccentrically placed and several small masses. Cleavage of these cells is unequal.

Some of the 48-hour sporocysts show a definite central cavity but in others no cavity could be seen. The cuticula around the outside of the body is considerably thicker than in the 24-hour sporocyst.

5-Day Sporocyst.—Between the second and fifth days the sporocyst and enclosed rediae increase very rapidly in size (Table 10). The central cavity becomes more distinct and the body walls become much thinner. At the two extremities the body is filled by a large number of cells, making the walls much thicker in these regions than they are laterally. In sectioned specimens the difference in thickness of these regions is not so evident as in living specimens. This is due to the fact that the developing rediae are uniformly placed in an undisturbed sporocyst and keep it more extended than in a sporocyst dissected from a snail. When fixed quickly the sporocysts do not contract, but living specimens usually contract at both extremities, forcing the rediae to the center of the body (Fig. 25), and consequently the two extremities appear to be very thick-walled. The number of rediae in 5-day sporocysts is variable but is never found to exceed 5. The size of the largest redia in each of 10 sporocysts of this age is shown in Table 10. No structures of the redia are recognizable at this stage in development.

10-Day Sporocyst.—A very few of the sporocysts in this experiment reached their maximum size in 9 days and rediae were found free in the tissues of the snail. The size of 10 sporocysts and the largest redia in each is shown in Table 10. The time required for the sporocyst to reach this stage of development during the winter months was very much longer. In the experiment begun on December 20, 1933, no free rediae were found in the snails until February 2, 1934, representing a difference of 35 days required to reach similar stages.

15-Day Sporocyst.—By the fifteenth day many rediae were found free in the snail, but as indicated in Table 10 only a small number of the sporocysts had reached their maximum size.

An occasional sporocyst was found in snails as long as 35 days after infestation, which in this experiment was after cercariae were being shed.

These sporocysts were usually located in the foot where conditions were perhaps not as favorable for growth as in other parts of the body. Others were found in the anterior margin of the mantle. Many authors have reported extensive migrations by the sporocysts of other species of trematodes, but the sporocysts of the present species were never found posterior to the anterior margin of the kidney. In most instances the sporocysts were found completely surrounded by an unbroken layer of cells produced by the snail, which indicates the relative immobility of the sporocyst.

The number of fully developed and developing rediae was never found to exceed 9 in a mature sporocyst (Fig. 35). Usually there are one or two ready to be liberated, while the remainder are in various stages of development. There is no birth pore in the sporocyst and the rediae can be liberated only by rupturing the body wall. The rediae most advanced in development are always located at the anterior end of the sporocyst, and it is this region of the body which is ruptured. A number of sporocysts were observed in which this rupture was evident. It was always at the extreme anterior end, and posterior to it the sporocyst was strongly contracted, causing the torn end to flare out. The constriction of the body prevents the less developed rediae from escaping. Thomas (1883:120) states that this constriction is maintained until the rupture is healed but this observation could not be confirmed. It is probable that the act of rupturing the body wall is initiated by the rediae, but observations made on sporocysts containing advanced rediae indicate that the sporocyst is an active participant in the process. When a redia is ready to emerge it moves or is forced into the anterior end by contraction of the body wall. The sporocyst then contracts behind it forcing it strongly against the anterior end of the body, and in this way apparently takes a part in rupturing its own body wall (Fig. 36).

Old sporocysts which have ruptured walls contain only a few rediae, some having been observed in which there were only 3 present. This fact, combined with the fact that they decrease rapidly in number in infested snails, points to the conclusion that each sporocyst will produce a definite number of rediae. For this species the number is probably 9.

Excretory System.—The excretory system of the sporocyst consists of the two original flame cells and their ducts present in the miracidium. The flame cells are readily visible at all times in the living specimens and it is quite easy to trace the ducts. These structures increase in size as the sporocyst develops, the flame cells reaching a size of 15 by 5 μ. The course of the ducts in the young sporocyst is exactly the same as in the miracidium (Fig. 25) but tends to become straighter as the sporocyst

becomes larger (Fig. 26). The course followed by these ducts depends quite naturally on the state of contraction of the individual and in some contracted old sporocysts the convolutions performed by them are very similar to those in younger specimens. The small bladder present at the end of each duct in the miracidium is also present in the sporocyst.

Shape.—The shape of the sporocyst as seen in sectioned material is ovoid at all stages of development. When young specimens are dissected out of the host into physiological salt solution they are capable of assuming a spherical shape, but the older specimens cannot contract to this extent, due to the presence of large rediae. They are able to contract the extremities strongly, and freed specimens usually have the posterior end more strongly contracted than the anterior end.

Muscles and Activity.—The circular muscles are strongly developed throughout the body, as evidenced by their activity, but the longitudinal ones seem to be less well developed. Very young specimens when freed from the host assume the spherical shape immediately, and subsequent movements are so slight as to be hardly noticeable. The fully developed sporocysts are relatively active. Their activity consists of slow contractions of the muscle layers which are too weak to produce any appreciable progression.

Appearance.—The fully developed sporocyst is visible to the unaided eye but cannot be readily distinguished from rediae or from small particles present in the water. The outside of the body is covered by a mucus which is evidenced by the ability of the sporocyst to cling to the bottom of a dish or to a slide and by the amount of débris which adheres to it. The cuticula on the outside of the body is thrown into fine transverse striations by the contraction of the longitudinal muscles, which gives the body a distinctly ridged appearance. The simultaneous contraction of the muscle layers at the anterior end of the body produces a knobbed appearance (Fig. 25). This condition is so characteristic of the living sporocyst that one is able to distinguish it from rediae and to orient it very quickly with the aid of the microscope.

Mature Sporocyst

The mature sporocyst is elongate, usually bluntly rounded at the two extremities, and circular in cross section. It is relatively simple in structure, consisting of a wall of variable thickness surrounding a central cavity (Fig. 30). The walls are composed of a thin cuticula, a layer of circular and longitudinal muscle, and an epithelial layer.

The cuticula is from 2 to 3 μ thick when measured in mature sectioned sporocysts, but when measured on living specimens of the same

age it appears to be from 5 to 6 μ thick, as seen in optical sections. This difference is due partially to the contracted state of the living specimens and partially to the fact that less accurate measurements can be made on living material. Immediately beneath this layer are the circular muscles, which can be seen distinctly. The longitudinal muscles were not seen, although they were looked for with a magnification of approximately 1500 diameters. The thickness of the combined layers is only 3 μ.

The epithelial layer consists of large vacuolated cells and small cells with a granular, deeply-staining cytoplasm (Figs. 27, 30). The large cells are more numerous than the others and comprise most of the body wall. They measure from 30 to 40 μ by 20 to 35 μ and contain nuclei which measure from 8 to 10 μ. A distinct chromatin mass, usually eccentric in position, is present in all of them. There are also many granular masses of chromatin scattered throughout the nuclei. The small cells measure approximately 18 by 9 μ and the diameter of the nuclei is from 6 to 7 μ. The chromatin of these nuclei has the same arrangement as that in the nuclei of the larger cells but is more dense. These small cells are readily distinguished from the larger cells by the difference in size and by their darker staining reaction. The distribution of these cells is very irregular. They may be located in contact with the muscle layer or scattered among the larger cells, although more of them were found in the posterior tip of the body than elsewhere.

That these small cells are probably germinal is indicated by their location and similarity in size and staining reaction to the cells of very young rediae (Fig. 30). Many workers have described similar cells in sporocysts of other species as germ cells.

Thomas (1883:115) in his description of the life cycle of *Fasciola hepatica* says:

> The contents of the sporocyst are formed by a number of very clear rounded cells, some of which are the germinal cells of the embryo or cells derived from them by division, others are formed by a proliferation of the epithelium lining the cavity of the sporocyst.

Looss (1896:187) states of the sporocyst of *Paramphistomum cervi:*

> Tandis que sur la paroi interne du sporocyst, on ne rencontre que rarement, ... des cellules germinatives normales, celles-ci se présentent amassées dans l'extrémité caudale où elles vont former un véritable épithélium germinatif.

Mathias (1925:50) describes two kinds of cells in the walls of the sporocyst of *Strigea tarda* which are similar to those present in the wall of the present sporocyst. The smaller of these he believes to be germ cells. Dubois (1928:63) describes similar cells irregularly dispersed in the body wall of the sporocyst of *Cercaria helvetica* v which he considers to be germ cells. Brooks (1930) in his detailed study of the germ cell cycle in 20 species of trematodes did not find any evidence to support the

theory that any cells in the epithelial layer of the sporocyst wall were germ cells. Price (1931:709) believes that the larger of the two types of cells found in the sporocyst wall of *Schistosomatium douthitti* are germ cells.

It is not my purpose to enter into the merits of the many conflicting viewpoints, but in the present material only 9 rediae are produced in each sporocyst, and I believe that the germ cells producing these are formed in the germinal tissue of the miracidium prior to its penetration into the snail host. If the small, deeply-staining cells present in the wall of the sporocyst are germ cells then the vast majority of them never produce rediae.

The rediae develop entirely enclosed by sporocyst tissue which divides the cavity of the sporocyst into compartments and in which the rediae remain until late in development. The fibers seen attached to developing rediae by Looss (1892:159) in the sporocyst of *Diplodiscus subclavatus* and by Price (1931:709) in the sporocyst of *Schistosomatium douthitti* are probably the same structures described here.

REDIA

Rediae belonging to the family Paramphistomidae have been described by a number of authors. Looss (1892) studied the development of the redia of *Diplodiscus subclavatus* and described the developmental stages and the mature redia in great detail. He also (1896) described briefly the rediae of *Paramphistomum cervi* and *Gastrodiscus aegyptiacus*. Cort (1915) gave a few details concerning the structure of the rediae of *Cercaria inhabilis* and *C. diastropha*. Faust (1919, 1919a) did the same for the rediae of *Cercaria frondosa* and *C. convoluta*. Sewell (1922) described rather completely the rediae of *Cercariae Indicae* XXI, XXVI, XXIX, XXXII. McCoy (1929) gave a brief description of the redia of *Cercaria missouriensis*. Beaver (1929) described the redia of *Allassostoma parvum*. Le Roux (1930) mentioned the fact that daughter rediae occur in the life cycle of *Cotylophoron cotylophorum* but gave no morphological details. Krull and Price (1932) described very briefly the redia of *Diplodiscus temperatus*.

These rediae belong to the subfamilies Paramphistominae Fischoeder 1901 and Diplodiscinae Cohn 1904. The rediae of *P. cervi*, *C. Indicae* XXVI, XXIX, XXXII, and *C. cotylophorum* belong to the subfamily Paramphistominae. These rediae are readily distinguished from those of the Diplodiscinae by the absence of lateral appendages. In general, the rediae of the Paramphistominae are smaller and possess a smaller pharynx and gut, although these characteristics are not of diagnostic value.

Development

Structure of the Redia.—The redia is much more complex in structure than the sporocyst. It possesses a well developed digestive tract consisting of a mouth, pharynx, esophagus, rhabdocoel intestine, and a large number of unicellular glands which are associated with it. The redia also possesses a more complex excretory system, a discernible central nervous system, and a birth pore.

Rate of development.—Development of the redia in the sporocyst is very rapid and at the time of liberation all the structures of the mature redia are present, with the exception of the birth pore. In the experiment under discussion the first rediae free in the body of the snail host were found 9 days after infestation.

The growth of the redia in the sporocyst was studied in an attempt to establish the chronological sequence of organ development and to determine the time of germ ball development. During the first 3 days the redia is spherical in shape and consists of numerous cells with indistinct boundaries which contain nuclei varying from 3 to 6 μ in diameter. On the fourth or fifth day the redia begins to elongate and the primordia of the digestive system appear. The smallest embryo with the primordia measured 68 by 45 μ (Fig. 42).

Digestive System.—The primordia of the digestive system consist of a group of centrally located cells whose cytoplasm is finely granular. These cells measure 10 to 12 μ in diameter and contain relatively large nuclei, 6 to 7 μ in diameter. Looss (1892:160) states that these cells produce a secretion which forces them apart, thus producing the lumen of the digestive tract. The cause of the separation was not determined in the present material, but the lumen is produced by a delamination of the primordial cells. As the embryo continues to grow the digestive primordia increase rapidly in size, extending from near the anterior end almost to the posterior end in embryos measuring 80 to 50 μ. In rediae of this size a small number of loosely organized cells which are destined to form the pharynx are present at the anterior end of the digestive tract. The lumen of the intestine becomes much more evident at this stage, being widest at the middle of its length. Anteriorly it is much narrower where it joins the pharyngeal cells.

Following this stage it rapidly assumes the appearance characteristic of it in the mature redia. The pharynx becomes definite in shape, a basal membrane develops around it and the intestine, and muscle fibers begin to develop in the pharynx. The development of muscles in the pharynx is accompanied by a breaking down of the cell membranes of the cells from which it develops. However, the nuclei of these cells remain dis-

tributed irregularly through it. In an embryo 106 by 55 μ, representing this stage in development, the pharynx measured 26 μ wide and 16 μ long and was located 14 μ from the anterior end of the body. The intestine does not increase in length to accompany the increase of body length. In the above specimen it terminated 27 μ from the posterior end.

Early in development, when the embryos have reached a length of approximately 90 μ, the cuticula lining the mouth cavity, pharynx, and upper part of the esophagus begins to form. Six cells at the anterior end of the intestine, which are designated as pharyngeal cuticula cells, grow forward through the lumen of the pharynx but do not entirely occlude it. The cells are united at their anterior ends, forming a cap which closes over the lumen (Fig. 44). As growth continues the cells elongate and their anterior ends approach the surface of the body. At this stage the cells measure 31 μ in length and 6 μ in width at the posterior end. At the anterior ends of these cells and near the surface the primitive epithelium covering the body grows inward around them until it reaches the anterior margin of the pharynx, where it seems to fuse with the inner surfaces of the cells in embryos approximately 100 μ in length. That this process is a result of growth and not of invagination of the anterior part of the body was demonstrated by study of serial sections of the embryo. The primitive epithelium was found to be intact over the entire surface of the body. That portion of the body lying directly anterior to the pharynx, represented by the nuclei in Fig. 44, is eventually sloughed and apparently forms a plug which fills the mouth cavity in slightly larger individuals (Fig. 49). The function of this plug is unknown.

At the time of the fusion of the primitive epithelium with the pharyngeal cuticula cells a substance is deposited in the surface cells next to the lumen of the pharynx. This substance, which forms the cuticular lining of a part of the mouth cavity, the pharynx, and a part of the esophagus, stains darkly in haematoxylin and is very difficult to destain. This characteristic indicates its extent in older forms, since the cuticula produced by the primitive epithelium does not stain so deeply. Accompanying the formation of the pharyngeal cuticula the nuclei of the cells producing it degenerate. The nuclei first become flat and elongate, the chromatin then forms a large central mass, and finally the nuclei disappear entirely, leaving a uniformly thin cuticula continuous with that of the body. The nuclei of the primitive epithelium degenerate also during its transformation into the cuticular covering of the body, which is concurrent with the formation of the pharyngeal cuticula.

Simultaneously with the formation of the pharyngeal cuticula, 6 other cells, which are designated as esophageal cells, become differentiated at

the anterior end of the intestine. These cells are broad at the base but become thin anteriorly at their junction with the pharyngeal cuticula cells (Fig. 43). The esophageal cells either produce a cuticula-like substance or are covered externally by the secretions of the pharyngeal cuticula cells, since they stain in a similar manner during the early stages of their development. Later this staining reaction disappears. However, the fact that this is cuticula is proved by the stiffened esophagus which projects prominently into the lumen of the intestine in contracted rediae.

In embryos 150 μ in length the formation of these structures is complete, the mouth is open but still contains the plug, and the entire embryo is still enclosed by sporocyst tissue (Fig. 40). The pharynx is 34 μ wide and 22 μ long, and the intestine, which is approximately 40 by 30 μ in embryos of this size, extends slightly past the middle of the body. The intestine consists of a single layer of distinct cells which measure 10 to 15 μ in diameter. Each contains a relatively large nucleus. The basal membrane which encloses both the pharynx and the intestine contains distinct ovoid nuclei early in development but they could not be found in the membrane in rediae of this size. Looss' (1892:161) observation that muscles develop in this membrane was not confirmed for this redia.

No rediae larger than 150 by 52 μ were found enclosed in sporocyst tissue, nor any which contained the plug in the mouth cavity. Apparently they break out of their individual compartments shortly after reaching this size and the mouth opens.

Looss' (1892:160-161; figs. 6, 7) description and figures of the development of the digestive organs in the redia of *D. subclavatus* show it to be very similar to that of the present redia. He did not observe a sloughing of the primary cuticula, which is produced by the primitive epithelium and pharyngeal cuticula cells, in the redia of his material, but he did observe indications of such a process in the redia of *Cercaria cystophora* (1892:161) after being born. He believes that a similar process occurs in the redia of *D. subclavatus* and that a secondary cuticula is produced by the underlying layers of the body wall. According to his observations neither the mouth nor the birth pore are open until after birth. In *C. cotylophorum* the mouth of the redia is open at least two days before birth but there is no indication of a birth pore. At this stage of development no indications of sloughing of the primary cuticula other than that previously mentioned were observed in the present material at any stage of development. Since no subsequent sloughing was observed it is believed that the primary cuticula is not sloughed at any time and that it forms the cuticula of the mature redia.

Germ Cells.—The germ cells appear simultaneously with the primordia

of the digestive system, that is in individuals approximately 60 μ in length. At this size living rediae appear as a homogeneous mass of cells surrounded by the primitive epithelium. However, in sectioned material the germ cells are obvious because of their size, position, and staining reaction. They were always found in the posterior region of the body, posterior or lateral to the digestive primordia (Fig. 42). The cells do not have definite boundaries, and the cytoplasm, which is finely granular, takes a deeper stain than the cells of surrounding tissue. They measure approximately 10 by 8 μ and contain nuclei which are 6 μ in diameter. There is no distinct central cavity in the young individuals but as the germ cells divide to form germ balls a small space becomes evident around each one. The smallest redia in which a definitely formed germ ball was found measured 85 by 39 μ. In an individual measuring 152 by 54 μ 4 germ balls were present. Usually there are 10 to 12 present when the redia is born. Early in the formation of the germ balls an occasional ovoid nucleus is observed near the periphery. These are the nuclei of the cells which are destined to form the primitive epithelium around the germ ball. These nuclei were observed in germ balls as small as 15 μ in diameter, but no definite retaining membrane is formed until they have reached approximately 30 μ in diameter. The retention of the germ balls in individual compartments is not very evident in the young rediae because of the thickness and irregularity of the body wall but this arrangement becomes very distinct in older specimens (Fig. 39).

Excretory System.—The earliest stages in the development of the excretory system were not observed. Looss (1892:161) states that in *Distomum ovocaudatum* he observed the excretory system of the redia in the 2-flame-cell stage; each cell opened separately at the posterior end of the body. In the present material no stage as early as this was observed. However, I believe that the excretory system becomes functional when the redia is approximately 75 μ in length. Small lateral tubules toward the posterior extremity were observed in embryos of this size but no flame cells could be distinguished. The smallest redia in which flame cells were observed was 105 by 84 μ. In this individual there were 2 on each side. The anterior pair is located lateral to the anterior end of the intestine, and the posterior pair is located in the extreme posterior end (Fig. 35). The excretory ducts on each side unite to form a common duct which expands to form a small bladder on either side shortly before reaching the excretory pore. In individuals of this size the excretory pore is located approximately one-third of the body length from the posterior end. A third flame cell is developed on each side when the redia is about 160 μ long, although specimens 135 μ long which possessed the

third cell were found occasionally. This cell develops near the excretory pore and its duct unites with the duct from the anterior flame cell (Fig. 46).

Nervous System.—The nervous system of the redia develops at the same time as the digestive and excretory systems. The central fibrous nerve mass is located dorsal to the esophagus, and the nerve cells completely surround it (Fig. 37). The region of the body in which it develops is filled by numerous cells at all stages of development and it is very difficult to determine when the nerve cells become differentiated. However, in rediae 100 μ long some nuclei have the appearance which is characteristic of the nerve cell nuclei in older forms. The nuclei are round, measure 4 to 5 μ in diameter, and contain numerous granular masses of chromatin which cause them to stain more darkly than other surrounding nuclei. The nuclei are scarce over the dorsal surface of the fibrous mass but are very numerous lateral to it. They extend to the ventral side of the redia and across the body ventral to the esophagus. The central fibrous mass is approximately 30 by 15 μ in rediae 150 μ in length. No nerves were observed to leave this central mass.

Salivary Glands.—The salivary glands which surround the anterior part of the digestive tract become differentiated in rediae slightly over 100 μ in length, being first observed in an individual which measured 104 by 52 μ. In this redia 6 cells were found which were considered to be gland cells. These glands are characterized by finely granular, deeply-staining cytoplasm and a relatively large nucleus which contains a few granular masses of chromatin (Fig. 34). There is also a concentration of chromatin at the nuclear membrane, while the remainder of the nucleus remains comparatively clear. The cells are drop-shaped with a long slender projection extending anteriorly. The cells are approximately 6 μ in diameter at their posterior ends, and the nucleus, which is located here, is 7 by 4 μ. The anterior extensions of the glands lengthen as the redia grows but do not reach the surface until the mouth cavity is being formed. In a redia about 150 μ in length 40 of these glands were found. Twelve of them were located around the pharynx, some lying anterior to it, while the remaining 28 were distributed around the esophagus and anterior part of the intestine. Looss (1892:161), Cort (1915: 23, 25), Sewell (1922:71, 77, 86), and Krull and Price (1932:9) have described very similar glands in other amphistome rediae.

Muscle Tissue.—The development of the muscular tissues is seemingly very slow. No movement is noticeable in rediae under 125 μ in length. At this size movement consists of very slow and weak contractions of the circular and longitudinal muscles. The individual muscle

layers could not be distinguished in rediae of this size, but the thickness of the combined layers is only 1 to 1.5 μ.

Body Wall.—The body wall in the young redia is similar to that of the young sporocyst. It consists of a very thin cuticula 1 μ thick, the muscle layer, and an inner epithelial layer which is from 10 to 15 μ thick in rediae developing in the sporocyst. The germinal tissue is located in the posterior extremity of the body, where it forms a mass which is approximately 30 μ thick.

Redia Prior to Liberation.—By the end of the seventh or eighth day after infestation the largest rediae are approximately 150 μ long and are similar in every way to mature rediae, except for their lack of a birth pore. The chronological sequence of organ development and germ ball formation is: (1) primitive epithelium, (2) digestive primordia, (3) germ cells, (4) excretory system, (5) nervous system, (6) gland cells, and (7) muscle development, as evidenced by movement. The sequence of organ development and the formation of early germ balls is very similar to that of the miracidium.

Liberation of Rediae—Rediae are found free in the central cavity of the sporocyst for one or two days before they break out into the body of the snail. During these days the only noticeable changes are an increase in the size and number of germ balls. Usually there are only one or two of these more advanced rediae present in the sporocyst at one time. The process of breaking out of the sporocyst was considered in the discussion of the sporocyst development.

The fact that the rate of development is unequal may explain why rediae of variable sizes are liberated by the sporocysts. Some rediae were found free in the snail host at a size of 169 by 52 μ, while on the other hand rediae as large as 225 by 58 μ were observed still in the sporocyst. The average size of 10 at the time of liberation was 188 by 56 μ. Looss (1892:162) found that the rediae of *Diplodiscus subclavatus* were freed when approximately 200 μ in length. At the time of their liberation the rediae contain from 10 to 12 germ balls, the largest of which are approximately 20 by 20 μ. In an individual which measured 225 by 67 μ the pharynx was 29 μ wide by 22 μ in length; the intestine was 71 μ long by 54 μ wide and terminated near the middle of the body length.

Birth Pore.—The rediae begin feeding upon the host tissues immediately after their release and migrate slowly into the liver and ovo-testis where they complete their development. The birth pore is the only structure which develops after they leave the sporocyst. It was first observed in a living specimen which measured 0.27 by 0.1 mm. In contracted specimens it appears as a small projection on the ventral surface of the

body, approximately 160 μ from the anterior end. Sewell (1922:71) found the birth pore of the redia of *Cercariae Indicae* XXVI to be situated to one side just behind the level of the pharynx, and in the rediae of *C. Indicae* XXIX he (p. 77) found it to be ventro-lateral one-fourth of the body length from the anterior end. Beaver (1929:16) found that the birth pore of the redia of *Allassostoma parvum* was dorsal to the anterior part of the intestine, and that it was visible only when cercariae were emerging through it. The central nerve mass of the redia is considered to be dorsal, and the flame cells, excretory ducts, and pore are considered to be lateral. By using the position of the excretory organs for orientation of the redia the birth pore is found to be ventral. In living specimens it can be seen only in a lateral view, and in sectioned material it is found ventral to the brain mass (Fig. 38). The time at which it opens was not determined.

Increase in Size.—The increase in size of the redia is very rapid. In the present experiment the largest redia ever found was dissected from a snail 10 days after infestation (Fig. 48). This specimen was 1.02 by 0.21 mm in size, the pharynx measured 55 by 55 μ, and the intestine was 160 by 84 μ. It contained 23 well-formed germ balls or cercariae, and others seemed to be developing at the posterior end of the body. The largest of the cercariae measured 90 by 65 μ. This individual was perhaps an aberrant form since the next largest specimen ever found measured 0.84 by 0.18 mm and contained only 14 cercariae. Apparently the redia reach their maximum size immediately before the birth of the first cercariae and then decrease somewhat in size as the cercariae are born. The redia in Fig. 33 was taken from the snail mentioned above and was the largest of the remaining rediae. It measured 0.55 by 0.12 mm, the pharynx was 48 by 50 μ, and the intestine was 105 by 64 μ. It contained 15 cercariae. The size of the 10 largest rediae found, the size of the pharynx, the position of the birth pore, and the number of cercariae developing in each is given in Table 11. Many rediae in all stages of development were constantly found in the snails used in this experiment, due to the fact that the sporocysts continued to produce rediae until the termination of the experiment, 35 days after infestation of the snails.

Mature Redia

There is no marked change in the appearance of the mature rediae from that of those just born. The pharynx and intestine increase in size but are much smaller in relation to body size than in young rediae. The pharynx of 10 rediae just liberated from the sporocyst averaged 32 by 27 μ, while the average size of this structure in the rediae, given in

Table 11, is 49 by 40 μ. The pharynx was never observed to become elongate, its greatest diameter always being the transverse one. The intestine, which reaches the middle of the body in young specimens, does not extend posteriorly more than one-fifth of the body length in mature forms.

TABLE 11.—MEASUREMENTS (IN MILLIMETERS) OF TEN MATURE REDIAE AND THE NUMBER OF CERCARIAE IN EACH

No.	Size of redia	Size of pharynx	Distance of birth pore from anterior end	Number of cercariae in each
1	1.01 x 0.22	0.055 x 0.050	0.180	23
2	0.94 x 0.18	0.050 x 0.048	0.174	14
3	0.77 x 0.21	0.050 x 0.042	0.165	18
4	0.67 x 0.13	0.046 x 0.046	0.147	12
5	0.62 x 0.12	0.046 x 0.034	0.189	10
6	0.60 x 0.17	0.042 x 0.042	0.178	12
7	0.59 x 0.17	0.046 x 0.042	0.162	15
8	0.58 x 0.15	0.046 x 0.046	0.155	14
9	0.57 x 0.17	0.046 x 0.046	0.170	16
10	0.55 x 0.12	0.048 x 0.040	0.148	15
Average	0.59 x 0.16	0.049 x 0.040	0.168	15

The excretory system is typical of all the amphistome rediae which have been described, with the exception of the redia of *Paramphistomum cervi* and *Diplodiscus subclavatus*. Looss (1892:161: fig. 10) found 3 or 4 pairs of flame cells in the redia of *D. subclavatus*, and in the mature redia of *P. cervi* there are 5 pairs of flame cells (1896:188). The flame cells in the mature redia of *C. cotylophorum* are located as in the young forms. However, the excretory pore is situated very near the middle of the body length, because of the posterior extension of the body caused by the developing cercariae (Fig. 46). The flame cells are approximately 12 by 5 μ and the ducts, which are only a little coiled, are 2 to 3 μ in diameter. The ducts from the anterior and posterior cells on each side unite slightly anterior to the middle of the body. The duct from the middle cell joins the duct from the anterior flame cell. The common duct formed by the union of the anterior and posterior ducts is approximately 50 by 10 μ in size. It expands to form a small bladder 20 by 30 μ which opens to the outside through a very small canal. The excretory pore when expanded measures 10 μ in diameter. The nervous system remains unchanged in the mature redia. The salivary glands increase in size as the redia grows. In a redia which was 0.35 mm long the cells measured 14 by 10 μ, being very conspicuous in sectioned material

because of this increase in size and a darker staining reaction. In old specimens which have passed maturity these cells stain lighter in haematoxylin stains and seem to be fewer in number.

The germinal tissue, which is located in the posterior extremity of the body, becomes exhausted in the older forms (Fig. 41). This exhaustion was found to occur in some rediae in this experiment 25 days after infestation of the snail, although there were many developing cercariae still present in them. This indicates that the number of cercariae produced by a single redia is very limited. In Table 11 the number present in mature rediae is shown to vary from 10 to 23, with an average of 15. This average doubtless is less than the number produced by each individual which is believed to be nearer 25. If this estimate is approximately correct, then the number of cercariae produced by each miracidium is 225, since each sporocyst produces 9 rediae. Naturally this estimate precludes the formation of daughter rediae. Takahashi (1928: 278) found that the sporocyst of *Paramphistomum cervi* produces 9 rediae, each of which gives rise to 20 cercariae, resulting in a total of 180 cercariae produced by a single sporocyst. Krull (1934:174) attempted to correlate his findings with those of Takahashi based on a total number of cercariae shed by each snail. He found that the average production of each of 11 snails was 152 cercariae; but since he allowed uncounted numbers of miracidia to infest his snails, no accurate estimate could be made as to the number of cercariae produced by a single miracidium. Consequently, he has no basis for his attempt at correlation with Takahashi's observations.

The body wall of the mature redia is very similar to that of the sporocyst. It consists of a thin cuticula covering the external surface of the body, a layer of circular muscles followed by a layer of longitudinal muscles, and a thin epithelial layer. The cuticula gives a white appearance to the redia and is wrinkled in low transverse ridges by contraction of the body. The cuticula appears to be 4 to 6 μ thick in living specimens, but in well extended sectioned rediae it measures 1.5 to 2 μ in thickness. The fact that it is no thicker in the older forms than in young rediae indicates that there are no additions to it by underlying cells, and this condition supports the evidence concerning the transformation of the primitive epithelium into the cuticula.

This study of the developing and mature redia demonstrates that the development of this stage in the life history of *C. cotylophorum* is very rapid. The chronological sequence of organ formation and the time of germ ball formation is very similar to the same processes in the miracidium. The birth of the redia occurs 9 to 10 days after the snail host is

infested. All the structures are formed except the birth pore. The average size of rediae at the time of liberation from the sporocyst is 0.188 by 0.056 mm. Following liberation the redia reaches its maximum size in 5 days and may contain as many as 23 cercariae, all of which are retained in individual compartments. The germinal epithelium is located in the posterior extremity of the body and probably becomes exhausted after 25 cercariae are produced. The digestive system consists of a mouth, a pharynx, an esophagus, and a rhabdocoel intestine. The excretory system is similar to that of other amphistome rediae, consisting of an anterior, a median, and a posterior flame cell and their ducts on each side of the body. The nervous system consists of a mass of fibrous tissue and many associated ganglion cells located principally in the dorsal region of the body in the esophageal region. The body wall is composed of an outer cuticula, a circular and a longitudinal layer of muscles, and an inner epithelial lining. The average size of mature rediae is 0.59 by 0.163 mm and the birth pore is located on the ventral surface 0.168 mm from the anterior end of the body.

DAUGHTER REDIA

Daughter rediae have been reported for only three species of amphistomes. Looss (1896:184) mentions that daughter rediae occur in the life cycle of *Gastrodiscus aegyptiacus* and that occasionally both rediae and cercariae were found developing in the same mother redia. Looss (1896:189) also found that in the life cycle of *Paramphistomum cervi* two and sometimes three generations of rediae were produced. Beaver (1929:17) in his work on the life cycle of *Allassostoma parvum* found that daughter rediae were produced in mother rediae in which the pharynx and intestine were larger in proportion to the size of the body than in daughter rediae. He found, too, that the mother redia possessed only one pair of appendages and that the body was greatly distorted.

Only a single mother redia was found in the present material although many infested snails were examined over a period of nearly a year. This one specimen was found in a snail on August 4, 1934, one month after it was infested. It was not observed until too distorted by pressure to make accurate observations. However, it contained three well developed and three developing rediae. In the more developed individuals the digestive and excretory systems were fully formed and one or two germ balls were present. In this same snail many young cercariae were found in the liver as well as many other rediae which contained developing cercariae only.

CERCARIA

Development

The cercaria acquires most of its structures while still within the redia, although some are represented by primordia only. In the experimental series under discussion mature cercariae were shed 32 days after the infestation of the snail. The first cercariae were freed from the rediae 15 days after infestation in a very immature condition. They were about one-third of the size of mature cercariae.

The first differentiation which occurs is the formation of the primitive epithelium, which does not develop until the germ ball or cercaria has reached a diameter of 30 μ. The appearance of the primitive epithelium is very similar to that surrounding developing rediae but it loses its cellular nature much more quickly than in the redia. No nuclei were distinguished in this layer in cercaria more than 60 μ in length.

The next structures to appear are the cystogenous gland cells. The first of these were found irregularly scattered through the body of a cercaria which measured 50 by 46 μ. These cells are easily recognized because of their size and characteristic appearance (Fig. 52). The cells are 8 to 10 μ and have nuclei 6 to 7 μ in diameter. A distinct deeply-staining chromatin body, 2 μ in diameter, is located in the nucleus. The remainder of the nucleus is finely granular while the cytoplasm is very transparent. The number of these cells increases rapidly as the cercaria develops.

Shortly after this stage in development the cercaria becomes distinctly ovoid, and at a length of 65 μ the primordia of the digestive and excretory systems appear. The excretory system consists of two lateral ducts which open separately at the posterior end of the body. Looss (1892:162) observed a similar condition in the developing cercaria of *Diplodiscus subclavatus* and was able to see flame cells at the internal ends of the ducts. In the present material no flame cells were seen prior to the birth of the cercaria. The digestive primordia consist of a group of cells located centrally near the anterior end of the body, in which lumina appear very early (Fig. 52). The most anterior lumen is that of the oral sucker and the most posterior that of the rhabdocoel intestine, which Looss (1892:163) considers as probably homologous to the intestine of the redia. The esophagus appears as a solid cord of cells connecting the oral sucker and the intestine. The number of cells entering into the formation of these primordia is much greater than in the redia. No cell boundaries could be distinguished but the number of cells present is indicated by the many small, closely packed nuclei.

Shortly after the formation of these structures a basal membrane in

which an occasional ovoid nucleus is located develops around them. The nuclei of this layer can be distinguished from those of the surrounding tissues by their position only. The ultimate development of this layer was not determined in the present material, but Looss (1892:165) states that these cells are derived from body parenchyma and give rise to the muscle layers surrounding these organs.

A mass of deeply-staining cells located immediately posterior to the blind end of the intestine at this stage in development represents the primordia of the male and female genital systems. The cells of this mass can be distinguished from surrounding cells by their position and staining reaction only, but by following them in their subsequent development their function is discovered.

In embryos of approximately 90 μ in length the tail is formed as a broad but short part of the body at the posterior end, which becomes set off rapidly from the body by ventral and lateral constrictions. The cells entering into the formation of the tail are similar in every respect to those of the body. As the constrictions deepen the excretory vessels in the tail become confluent, but retain their separate openings, which become lateral in position because of the narrowing and lengthening of the tail (Fig. 57). The union of the excretory ducts occurs first in the tail but continues into the posterior part of the body for a short distance. As a result of this fusion the excretory system consists of a tail duct with its two lateral openings and a lateral duct on each side of the body. At the junction of the tail duct and the two lateral ducts another pore is formed in the median dorsal line of the body. This is the pore of the future excretory bladder in both the mature cercaria and the mature worm. This pore is formed prior to the birth of the cercaria.

While these changes are taking place in the excretory system the digestive system is becoming complete. The rhabdocoel intestine bifurcates and a mass of cells is formed at the end of each fork which grows laterally and posteriorly on each side. These cells are the primordia of the caeca (Fig. 55). As the caeca elongate a small lumen is formed in them. They develop dorsal to the excretory ducts.

At this stage of development the cuticula-producing cells of the oral sucker still retain their nuclei and the mouth is not yet open. The lumen of the esophagus is present and the esophagus is lined by a thin cuticula continuous with that of the oral sucker. The cuticula lining both the esophagus and the oral sucker is thrown into longitudinal folds which are continued a short distance anterior to the oral sucker. It is possible that the mouth opening is formed in the cercaria as in the redia but no observations were made on the details of this process. However, the mouth opening is formed before the cercaria is born, being similar in

this respect to the redia, but the nuclei of the cuticular cells are retained until after birth. Looss (1892:164) assumes that the primary cuticula is sloughed by the cercaria as in the redia but he made no observations on this process. In the present material no sloughing was observed and it is believed that the primary cuticula produced by the primitive epithelium and the cells lining the oral sucker is retained throughout the life of the individual.

Simultaneously with the formation of the tail the nervous system and eyes appear. The nervous system at this stage of development consists of a small ganglion on each side of the esophagus. These ganglia are connected by a commissure passing dorsal to the esophagus immediately posterior to the primordium of the oral sucker. Several small nerves can be traced a short distance from each of the ganglia (Fig. 57). Many nuclei were observed arranged in a close series which completely encircle the ganglia and commissure clearly delimiting them from the surrounding parenchyma cells. Looss (1892:164) described a similar nervous system for the developing cercaria of *Diplodiscus subclavatus* and was able to trace the nerves much farther than could be done in the present material. At the time of the formation of the nervous system and the tail the cells producing the eyes become evident. The pigmented part of each eye is produced from a single cell located laterally above each ganglion. These cells could not be distinguished from the surrounding cells prior to the formation of the pigment. After the formation of the pigment the cells are conspicuous, having the appearance shown in Fig. 64. At this stage in development the cell measures 10 to 12 μ in diameter and the nucleus 6 μ. The nucleus contains a large central chromatin mass 3 μ in diameter. Looss (1892:165) observed three cells which contributed to the eye formation in the early developmental stages of the cercaria of *Diplodiscus subclavatus*. As he pointed out, one cell produced the pigmented part and the other two the lens of the eye. These latter two cells were not identified in the present material prior to the birth of the cercaria and then were recognized in sectioned material only (Fig. 65). These cells lie against the cuticula, between it and the pigment cell, and seem to give rise to a refractive substance which fills the space in the pigment cone. The arrangement of these cells was determined in cercariae which had a body length of 130 μ.

Thus in the cercaria the following structures can be identified before it leaves the redia (in order of appearance): primitive epithelium, cystogenous glands, excretory and digestive primordia, reproductive tissues, nervous system, pigment cells of the eyes, and the tail. The only structure which develops after birth and of which there is no indication before birth is the acetabulum.

Birth occurs shortly after the formation of the tail but the size at which the cercariae are born is not uniform. The approximate size of the body is 120 by 55 μ and of the tail 37 by 33 μ. The cercaria is capable of very weak movement which is sufficient to break the enclosing strands of tissue but the process of birth was never observed. The mouth is open and the caeca which extend the length of the body are provided with a small lumen, and so it is possible for the cercaria to begin feeding at once. Immediately after birth the eyes rapidly become more prominent and the other structures of the body also increase rapidly in size (Fig. 56); the primordia of the reproductive systems divide, forming two large masses which remain connected by a small cord of cells.

The acetabulum first appears as a solid mass of cells at the ventro-posterior surface of the body in cercariae approximately 140 by 65 μ. This mass projects prominently and measures about 45 μ in width and 30 μ in length. A lumen becomes conspicuous in the acetabulum when the cercaria has reached a size of 190 by 75 μ. At this stage the acetabulum appears as a wide flat mass of cells with a small concavity near its center. It is 50 μ wide and 20 μ thick.

Shortly after birth the eyes acquire a very heavy pigmentation and their characteristic oval shape which is retained until the cercaria is 150 μ in length. Pigment then begins to grow out from the eyes in lateral finger-like projections which completely encircle the body. A short time later projections are formed at the posterior surface also. At the same time the eyes become surrounded by a solid mass of pigment which entirely obscures their original outline (Fig. 61). As the growth of the pigment continues it is arranged in an irregular dendritic pattern over the dorsal and lateral surfaces of the body (Fig. 61). The branches break up into small irregularly arranged patches which remain attached to each other by very small strands of pigment. A short time before maturity is reached only a few large branches of pigment, which extend laterally and posteriorly from the eyes, are present (Fig. 59). The eyes assume their original shape but remain connected by a conspicuous band of pigment. It is possible that these later concentrations of pigment lie directly above the large nerves of the body as suggested by Sewell (1922:75). In the mature cercaria all of the pigment is uniformly distributed, there being no definite concentrations into lines or large patches as in the developing forms. Consequently the eyes, which retain their pigmentation, become very conspicuous.

The pigment is entirely superficial in position, being located immediately beneath the cuticula with the exception of small granules which are scattered throughout the body. The concentration of pigment on the

dorsal and lateral surfaces is much heavier than on the ventral surface, but no area was found entirely devoid of pigment.

Simultaneously with the formation of the acetabulum the two lateral excretory ducts become united by a cross connection located across the middle of the body near the dorsal surface. The lateral excretory ducts pass outward and forward from their union with the caudal duct, then turn forward and inward and at their most inward position the cross connection is formed. From this point they turn outward and forward and pass ventral and mesial to the eyes to reach the sides of the oral sucker. Here they turn sharply and pass back toward the posterior end of the body. In cercariae between 150 and 175 μ in length a small median anterior diverticulum is formed on the cross connection and a lateral diverticulum grows out from each lateral duct immediately posterior to the eyes. The excretory system is shown in Fig. 50.

As has been stated previously an excretory pore is formed dorsal to the union of caudal and lateral excretory ducts prior to the birth of the cercaria. The excretory duct of the bladder is lined for a considerable distance by cuticula, which makes it evident that the pore is formed by an invagination of the body wall which comes in contact with the excretory ducts at their union. As the cercaria increases in length the posterior ends of the lateral ducts become situated more posteriorly. This change in position is slight but necessitates an anterior extension from their point of union to the excretory pore. At first, in cercariae of not over 0.2 mm in length, this anterior extension is no wider than the ducts, but as growth continues it assumes the appearance of the bladder or excretory vesicle in the mature cercaria. The lateral ducts empty at all stages of development into the posterior lateral margins of the bladder. The caudal excretory duct opens into the excretory duct from the bladder (Fig. 45). Consequently, the bladder is formed by first an extension and later an expansion of the ends of the lateral excretory ducts at their point of union.

The cystogenous glands which increase in number as the cercaria develops begin to produce cystogenous rods or granules when the cercaria is approximately 140 μ in length. Previous to this stage in development the cercaria stains very deeply in both toto mounts and sectioned material, but with the formation of the cystogenous granules this staining reaction largely disappears. This is due to the fact that the granules are very difficult to stain. The increase in number of these granules is so rapid and the area they occupy is so great that in cercaria of 200 μ in length there is comparatively very little tissue in the body. The body parenchyma is almost completely obliterated, only an occasional nucleus surrounded by a small amount of cytoplasm being found. The cystogenous cells are

round and vary from 13 to 21 μ in diameter when filled with the granules. The rod-shaped granules are arranged in parallel series in the cells and measure 12 by 4 μ.

All of the structures of mature cercariae are present but not fully developed in individuals which measure approximately 0.175 by 0.09 mm. The structures continue to increase in size but marked changes occur only in the pigmentation, the size of the excretory bladder, and the size of the tail. The changes occurring in the first two have been discussed previously.

In its early stages of development the tail consists of a dense mass of cells set off from the body. It is much broader than long when first formed and at the time of the birth of the cercaria the dimensions are practically equal. It is also capable of very slight movements which consist of slow contractions and extensions. It becomes longer than wide immediately after birth, and the length increases rapidly. Both the body and tail of the cercaria are subject to considerable variation in size in older individuals, but in well extended fixed specimens the tail and body become approximately equal in length when both are about 0.2 mm long. Three or four days before maturity is reached the tail is sufficiently strong to enable the cercaria to swim for brief intervals. Such cercaria will swim for a short time and then apparently attempt to encyst but cannot. In cercaria of this age the tail is approximately twice the length of the body.

The number of cells comprising the tail appears to remain unchanged. They are very small and numerous in the young stages, as indicated by the nuclei, but become large in mature individuals (Fig. 54), giving the impression that the cells increase in size but not in number as the tail grows.

Mature Cercaria

Age at Birth.—Mature cercariae escaped from the snails in this experiment 32 days after infestation. The development of the cercaria when compared to that of the redia is very slow. It will be recalled that the first rediae were freed from the sporocyst in 9 days and at the time contained developing cercariae 5 days old. The cercariae continued to develop in the redia for another 5 days before the first of the cercariae were born at an age of 10 days. Following their birth these oldest cercariae developed for another 22-day period in the liver of the snail before they made their escape.

Size.—Because of their size and heavy pigmentation the free-swimming cercariae are readily distinguishable while swimming if they are placed over a white background. The cercaria is extremely variable

in form, and since it is constantly active exact measurements are hard to secure. When fully extended the body may be three times its width and very flat. When fully contracted the width is slightly greater than the length and the body may be one-half as thick as it is wide. When fixed in warmed Bouin's or corrosive sublimate fixatives the body contracts still more. In the contracted state the anterior end of the body is pulled ventrally.

TABLE 12.—MEASUREMENTS (IN MICRONS) OF TEN CERCARIAE EXTENDED, CONTRACTED, AND FIXED

No.	Body measurements			Tail measurements		
	Extended	Contracted	Fixed	Extended	Contracted	Fixed
1	302 x 151	151 x 235	168 x 184	670 x 47	352 x 100	336 x 84
2	302 x 252	201 x 201	252 x 184	655 x 60	302 x 84	369 x 84
3	336 x 168	168 x 252	176 x 189	672 x 50	420 x 84	378 x 71
4	386 x 117	189 x 231	218 x 184	588 x 50	302 x 84	554 x 67
5	403 x 108	186 x 240	218 x 184	630 x 50	302 x 84	554 x 67
6	420 x 134	235 x 268	235 x 210	588 x 60	319 x 84	420 x 71
7	431 x 156	245 x 303	216 x 216	621 x 45	312 x 89	353 x 55
8	451 x 184	196 x 235	176 x 196	686 x 58	294 x 98	333 x 59
9	460 x 156	196 x 274	187 x 187	706 x 54	392 x 89	460 x 54
10	490 x 134	235 x 314	319 x 268	672 x 70	470 x 100	420 x 84
Average	398 x 153	200 x 252	236 x 200	668 x 55	346 x 89	417 x 69

The tail size varies considerably also. When fully extended it may be approximately twice as long as when contracted, but when fixed it is usually slightly longer than when contracted.

The measurements made on 10 cercariae when extended, contracted, and fixed are given in Table 12. When the average length of the body and tail of the 10 are combined the total length of the cercaria is 1.066 mm when fully extended and only 0.546 mm when fully contracted. The same measurement on fixed specimens was 0.653 mm.

Body Wall.—In contracted specimens the surface of the body is covered by a thin cuticula in which is seen a series of clear longitudinal lines running parallel to each other. Sewell (1922:75) saw similar lines in *Cercariae Indicae* and thought that they might denote longitudinal muscle fibers. However, since these lines are superficial in position, large and distinct, and can be seen only in living contracted specimens (not being seen at all in toto mounts or sectioned material) it is believed that they are only folds in the cuticula.

Pigment.—The pigment of the body is distributed over the entire body in a solid layer which is about 3 times as thick on the dorsal and

lateral surface as it is ventrally. A few pigment granules are scattered irregularly throughout the body (Fig. 58).

Eyes.—The eyes are located far forward, lateral to and immediately posterior to the oral sucker (Fig. 51). They are conical in shape with the base located immediately beneath the cuticula and with the apex directed ventro-posteriorly in the body. The base is surmounted by a clear refractile lens in which are two nuclei representing the cells from which the lens is formed. The nuclei measure approximately 7 by 5 μ and each contains a small distinct chromatin body. The cone is 30 μ long and 18 μ in diameter at its base. When the cercaria is swimming the anterior end of the body is drawn ventrally, leaving the eyes located at the most anterior and dorsal point of the body.

Acetabulum.—The acetabulum in the relaxed condition in living cercaria measures 65 μ in diameter. In contracted, fixed, and sectioned material the acetabulum is very much smaller. It measures 43 μ in diameter under such conditions.

Digestive System.—An accurate study of the digestive structures cannot be made in either living cercaria or toto mounts because of the heavy pigmentation and the cystogenous glands. The extent of the esophagus cannot be determined and the caeca are not visible in living specimens. In toto mounts these structures can be seen indistinctly. The oral sucker is terminal and usually ovoid in shape, its length slightly exceeding its width. Its average size in living cercariae is 47 μ in length and 46 μ in width; in fixed sectioned material the average size is 32 by 32 μ. When the oral sucker is retracted a distinct oral cavity is formed which is lined by smooth cuticula.

The esophagus originates near the ventral surface of the oral sucker and passes dorsally and posteriorly (Fig. 58). It bifurcates posterior to the eyes and in the dorsal part of the body to form the intestinal caeca. It is lined by a cuticula continuous with that of the oral sucker. The walls of the esophagus are thin at its origin but increase in thickness posteriorly. There is no definite sphincter muscle at its termination but the increase in thickness gives it a bulbous appearance. Deeply-staining glandular cells, the esophageal glands, completely surround the esophagus throughout its length (Fig. 47).

The caeca are small, measuring 15 μ in diameter, and terminate above the anterior margin of the acetabulum.

Nervous System.—No parts of the nervous system can be seen in living specimens or toto mounts and only the larger parts can be traced through sections. The central nervous system consists of two ganglia connected by a transverse commissure. The ganglia are located directly

beneath the eyes and the commissure passes across the body dorsal to the esophagus, immediately posterior to the dorsal surface of the oral sucker. The ganglia are approximately 15 μ in diameter and the commissure is 9 μ in diameter in the median line of the body. Each ganglion gives rise to several nerves. Three were found to pass anteriorly and terminate on the body surface lateral to the oral sucker, two being ventral and one dorsal. Another nerve passes dorsally and terminates in contact with the inner end of the eye (Fig. 66). A posterior nerve passes from the ganglion to the ventral surface of the caecum on the same side where it disappears from view. The ganglia, the commissure, and the nerves are entirely enclosed by numerous small nuclei which probably represent nerve cells.

Reproductive System.—The primordia of the male and female reproductive systems are distinguishable in the mature cercaria. These primordia consist of two large groups of cells connected by a single cord of cells (Fig. 58). The most posterior mass which represents the ovary, Mehlis' gland, and testes is located immediately dorsal to the anterior margin of the acetabulum. It consists of numerous deeply-staining cells and nuclei. This mass may vary from 22 to 29 μ by 16 to 20 μ in contracted specimens. From it a cord of cells passes anteriorly in the center of the body which connects with the second large group of cells located immediately posterior and slightly ventral to the intestinal bifurcation. From its ventral side a cord of cells passes ventrally, terminating against the surface of the body. Its point of termination marks the position of the genital pore but no opening could be found in the cercaria. The cells of these primordia are entirely similar.

Excretory System.—The excretory system of the mature cercaria is essentially the same as in the immature cercaria. The presence of the heavy pigmentation and the cystogenous glands makes it impossible to determine the flame cell pattern or the position of the smaller excretory tubules. Sixteen flame cells were found in developing cercariae irregularly distributed but principally around the acetabulum and oral sucker prior to the spreading of the pigment to these regions. Consequently, only the larger units are described here.

The excretory bladder is located dorsal to the acetabulum and the posterior genital complex (Fig. 58). A short cuticula-lined duct passes from its anterior end dorsally to the excretory pore located in the dorsal median line above the anterior margin of the acetabulum. In extended cercariae there may be a slight change in these relationships. In such specimens the duct passes dorso-anteriorly from the bladder and the excretory pore may be as much as 10 or 15 μ anterior to the acetabulum. Two large excretory ducts join the posterior lateral margin of the bladder. From

this point they pass outward and forward for a short distance and then turn inward. Slightly posterior to the middle of the body the cross connection previously described passes across the median line and sends off a small median diverticulum. The main ducts are ventral to the caeca but the cross connection passes dorsally and the diverticulum is located near the dorsal surface of the body a short distance posterior to the intestinal bifurcation. No tributaries were seen emptying into this diverticulum. Anterior to the cross connection the main ducts turn outward and then turn inward again posterior to the eyes. They pass forward ventral to the eyes and ganglia to reach the sides of the oral sucker. Here they turn sharply and pass back to the posterior end of the body, terminating near the acetabulum. Diverticula are formed on the main ducts also immediately posterior to the eyes. As in the median diverticulum no ducts were seen opening into them.

The main ducts from the bladder to the oral sucker, the cross connection, and the diverticula were filled with excretory granules varying from 2 to 10 μ in diameter. The smaller granules were located in the extremities of the ducts while the larger ones were located in the main ducts near the center of the body.

The wide caudal excretory tube joins the excretory duct just before it reaches the pore. It passes backward through the center of the tail and enlarges to form a small bladder at its posterior end a short distance from the end of the tail. From this enlargement a short duct passes posteriorly and laterally on each side to reach the surface. These ducts are the ends of the two original excretory ducts of the very young cercaria but the presence or absence of an opening for these was not determined for older ones.

Free-Living Cercariae.—It was observed that these cercariae escaped from the infested snails during a definite period each day and at no other time unless artificially stimulated. The time and numbers of cercariae escaping from individual snails are shown in Table 13. The snails used in this experiment were infested on May 5, 1934, and began shedding cercariae on June 5, 1934. There were 35 snails in this group, all of which were shedding cercariae at the same time, but only 15 were used to illustrate the periodicity of shedding.

Five snails were placed in individual finger bowls which contained a small amount of water and a piece of lettuce. The size and pigmentation of the cercariae makes them easy to see, especially when placed over a white background. It had been observed previously that the snails would shed cercariae at any time if they were kept out of water for as long as 24 hours and then put back into water. The first snails used in this experiment were taken from an aquarium (in which water was present at

all times) at 8:00 A.M. on June 14 and placed in the finger bowls. The cercariae began escaping a short time after 11:30 A.M. and continued to escape until 5:30 P.M. The snails were under observation continuously and the cercariae were removed as they emerged. Group 1 of Table 13 shows the results of this experiment. The snails were kept under observation in the bowls until 2 A.M. June 15 but no more cercariae escaped until about noon of the following day. A similar experiment was made with 5 other snails on June 15 and June 19. The results indicate definitely that cercariae escape in largest numbers during the brightest hours of the

TABLE 13.—SHOWING PERIODIC SHEDDING OF CERCARIAE BY THREE DIFFERENT GROUPS OF FIVE SNAILS EACH

Time	Group I					Group II					Group III				
	June 14, 1934					June 15, 1934					June 19, 1934				
10:30-11:30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11:30-12:30	55	5	104	0	5	310	7	32	32	14	126	125	18	0	138
12:30-1:30..	4	88	101	0	0	9	170	4	16	23	39	45	0	0	4
1:30-2:30...	0	0	0	90	0	21	46	2	8	1	14	0	0	0	0
2:30-3:30...	0	27	0	1	0	1	2	0	0	0	16	0	0	0	0
3:30-4:30...	0	1	32	4	0	0	0	0	0	0	1	0	0	1	0
4:30-5:30...	0	0	12	0	0	0	0	0	0	0	5	0	0	0	0
5:30-6:30...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total....	59	119	249	95	5	341	225	38	56	38	201	170	18	1	142

day. June 15 was a rainy day and much darker than either June 14 and 19, and cercariae escaped through a period of 3 hours only, while on the other days which were very bright this period increased to 6 hours in some instances. This response of the cercariae to light is demonstrated by free-swimming cercariae also. Immediately after escaping they collect in the dish where the light is most intense, and in a short time begin to encyst at the surface of the water. If vegetation is placed in the dish the majority of the cercariae encyst on the part at which the light is most intense. However, they select the vegetation for encystment in preference to the glass even though the light is less intense near it. This response to light is further demonstrated by the fact that cercariae make their escape at any time of the day if the snails are placed directly beneath a strong light. This response is most easily demonstrated in the following manner. If a number of cercariae are drawn into a pipette which is then held perpendicular to the edge of a lamp shade covering a lighted electric lamp with the pipette exposed to the light, the cercariae move back and forth in it. If the pipette is then drawn past the shade so that the upper end is darkened the cercariae move into the lighted area and never leave

it for more than a few seconds. By continuing to raise the pipette the cercariae can be concentrated at the end of the pipette. This method of concentration is rapid and useful in securing large numbers of cercariae in a minimum amount of water.

The cercariae are active swimmers but do not swim for long periods. After emerging from the snail they swim in a small circle for a short time and then proceed to the most illuminated part of the dish. They swim for a few seconds and then drop to the bottom of the dish or come to rest on small bits of débris or vegetation for a short time. At the sides of the dish they swim at the surface briefly then settle slowly to the bottom but are soon back at the surface again. This activity may continue for as long as an hour if there is no vegetation on which to encyst but none were found to remain unencysted for longer periods unless constantly disturbed. If vegetation is present some cercariae begin to encyst at once but others require as long as 30 minutes. They encyst readily on many kinds of vegetation but lettuce was used in most instances. If cercariae are place in the hollow of a lettuce leaf they collect in the small folds of the leaf but always on the side turned toward the light.

The number of cercariae escaping from the snails (Table 13) was observed to vary considerably. If a snail produced a large number of cercariae in one day, it was 8 days before any more escaped in some instances. However, the time interval may be as short as one day, but the number emerging on the second day is always small. That this variation is not due to a loss of infestation was demonstrated by dissecting some of the snails shown in Table 13 after an interval of 4 or 5 days during which no cercariae were shed. In every snail examined many developing rediae, mature rediae, and developing cercariae were found in large numbers.

Cort (1922:183) observed that cercariae escaped at definite periods each day and that these periods remained the same for specific cercariae but differed for different species. He also observed that there was considerable variation from day to day in the numbers produced. Krull and Price (1932:20) observed that the cercariae of *Diplodiscus temperatus* escaped at any time of the day but that largest numbers escaped between 11:00 A.M. and 5:30 P.M. They also observed that there is a very definite heliotropic response in these cercariae. Krull (1934:175) found that the cercariae of *Cotylophoron cotylophorum* escaped from the snail host between 8:30 A.M. and 1:00 P.M., the peak being about 8:30 A.M. This observation shows that the cercariae escaped periodically, but the time at which the largest numbers are shed is quite different from the findings in the present observations on the same species. Krull also found that these cercariae occasionally escaped at other hours of the day.

Krull and Price (1932) and Krull (1934) noted that the number of cercariae escaping from the snails increased the length of the period of escape. That this was not the case in the present experiments can be seen from the results shown in Table 13. Several instances are shown in which a larger number of cercariae escaped in less time than that required for the escape of smaller numbers from other snails.

The length of time snails remain infested was not determined. However, the time would depend on the rate of development of the various stages. In the present work snails infested during the coldest months of the year shed cercariae only after 91 days, and in the warmest months cercariae were shed 30 days after infestation of the snails. That a definite number of rediae is produced by each sporocyst and a definite number of cercariae is produced by each redia has been pointed out. Consequently the snails do become free of infestation but only after a prolonged period. Under optimum developmental conditions the sporocyst remains productive for fully 30 days, the redia for another 30 days, and the cercaria requires 22 days to develop after leaving the redia. Thus under theoretical optimum conditions snails remain infested for at least 78 days and probably remain infested under natural conditions for periods ranging from 4 to 6 months, which is doubtless equal to or exceeds the life of the snail. Krull (1934:175) was able to keep infested snails alive for 6 weeks after cercariae began to escape, 36 days after infestation. The number of days these snails remained alive after infestation, 76 days, is very close to the theoretical time limit they will remain infested under optimum conditions. Krull's work was done in the summer months of May, June, July, and August which may be considered as the optimum developmental period for the parasite.

Discussion of Previously Described Amphistome Cercariae

Sixteen species of amphistome cercariae have been described, two of which have been considered doubtful. Cary (1909:604-607) described an amphistome cercaria which he considered to be that of *Diplodiscus temperatus*. However, Cort (1915:23-30) has pointed out that Cary was mistaken in considering the cercaria he studied as being that of *D. temperatus*. Ward (1916:17-19) described *Cercaria gorgonocephala* and expresses his own uncertainty as to its exact systematic position, and as it is not closely related to the cercaria of *C. cotylophorum* it needs no further consideration here.

Looss (1892:162-166) described in detail the cercaria of *Diplodiscus subclavatus*. Later he (1896:185-191) described the cercariae of *Paramphistomum cervi* and *Gastrodiscus aegyptiacus* (1896:177-185). His

conclusions in the latter case, however, rest on the structural comparison of the cercaria and the adult.

Cort (1915:24) described *Cercaria diastropha* and *C. inhabilis* and called attention to the fact that the 5 species of amphistome cercariae then described belong to two subfamilies of the Paramphistomidae. He assigned the cercaria of *Paramphistomum cervi* to the subfamily Paramphistominae; and *Cercaria diastropha* and *C. inhabilis,* the cercaria of *Diplodiscus subclavatus,* and that of *Gastrodiscus aegyptiacus,* he assigned to the subfamily Diplodiscinae. Sewell (1922:66) accepted Cort's classification and added 3 new species, *Cercariae Indicae* XXVI, XXIX, and XXXII to the former group; and *C. frondosa* Cawston (1918), *C. corti* O'Roke (1917:165-180) and a new species *C. Indicae* XXI, to the latter. Sewell omitted *C. convoluta* Faust (1919:172), but Faust states that it probably belongs to the Diplodiscinae.

Sewell (1922:67, 80) prepared a key for the separation of two distinct groups for which he proposes the names "Pigmentata" and "Diplocotylea." He assigned the cercaria of *Paramphistomum cervi, Cercariae Indicae* XXVI, XXIX, and XXXII to the former group, and the other cercariae mentioned above he assigned to the latter group. To this latter group, Diplocotylea, McCoy (1929:200) added *Cercaria missouriensis.* Beaver (1929:20) described the cercaria of *Allassostoma parvum* and assigned it to the Diplocotylea also, and he points out that *A. parvum* belongs to the subfamily Schizamphistominae Looss 1912. He further points out the mistake made by Cort (1915:24) in assigning amphistome cercariae to subfamilies based on larval characteristics only, since the diagnostic characteristics of amphistomes are restricted to characters which are not present in the larval forms. His conclusion that only Sewell's classification can be rightly used is considered to be entirely correct.

C. cotylophorum belongs to the Paramphistominae, so that the present cercaria may be assigned to that subfamily. The cercaria possesses all of the characteristics of the "Pigmentata" and can be assigned to that group also.

This cercaria can be readily distinguished from that of *P. cervi* by its much smaller size, shorter tail, smaller suckers, and particularly by the presence of the evaginations from the excretory vessels. These evaginations are lacking in the cercaria of *P. cervi.*

Cercariae Indicae XXVI differs from the present cercaria in that *C. Indicae* is very much larger; the suckers are nearly twice as large; the esophagus is narrow without any trace of a pharynx or sphincter muscle; the genital system is well developed and shows a differentiation into the

respective organs, and the vitelline glands are well developed. In view of these differences there can be no doubt that the two cercaria are entirely different.

Cercariae Indicae XXIX presents fewer differences than the two above but can be distinguished from the present cercaria by a few characteristics of diagnostic value. This cercaria is slightly larger than the cercaria of *C. cotylophorum* yet possesses suckers almost twice as large; there is no thickening of the circular muscle layer around the esophagus, and the "anlage" of the vitelline glands are present. Consequently, these two cercariae must be considered as different species.

The only other cercaria assigned to the "Pigmentata" group is *C. Indicae* XXXII. This form is readily distinguished from the present cercaria also. *C. Indicae* XXXII is slightly larger and possesses an oral sucker 3 times as large, and an acetabulum more than twice as large as these structures are in the cercaria of *C. cotylophorum.* It differs also in possessing a very long esophagus with a well marked sphincter muscle at its posterior end, and in that the excretory pore opens posteriorly.

It is quite evident from a comparison of these forms that the cercaria of *C. cotylophorum* has not been described previously. Le Roux (1930: 247) mentions finding an amphistome cercaria in a snail, *Bulinus schakoi,* which he considers as practically identical with *C. frondosa* Cawston as described by Faust (1919:172), and which he thinks is the cercaria of *C. cotylophorum.* He did not describe it, however, and it is impossible to determine what cercaria he found. Faust describes *C. frondosa* as having pharyngeal pockets and does not describe the cross connection between the excretory ducts which is characteristic of the group to which the cercaria of *C. cotylophorum* belongs. The possession of pharyngeal pockets and the absence of the connecting duct clearly places *C. frondosa* in the "Diplocotylea" group. Consequently, if the cercaria found by Le Roux were *C. frondosa* it was not the cercaria of *C. cotylophorum.*

METACERCARIA

Cercariae remain free-swimming from 10 minutes to an hour after escaping from the snail, but the usual time for them to remain unencysted when vegetation on which to encyst is present is from 20 to 30 minutes. When encystment begins the cercaria actively elongates and contracts the body while the tail is beating violently. This movement alternates with one in which the body is thrown from side to side by the movements of the tail as in swimming, but no progress is made. At the same time the cystogenous material is breaking down and flowing out all over the body surface. The cuticula seems to loosen and the body contracts until a con-

siderable space is left between it and the cuticula. The cystogenous material collects on this membrane, producing at first a roughened surface both externally and internally. The cercaria is constantly in motion during this process, twisting the body from side to side. These movements smooth the internal wall of the cyst and distribute the cystogenous material evenly. The tail continues to beat rapidly, and as the cyst is formed it becomes loosened from the body but may remain attached to the cyst wall for some time. When freed from the cyst it swims away and continues to swim for as long as 8 hours. The process of encystment is usually complete after 20 minutes but the metacercaria may continue to contract from side to side for a much longer period.

The completed cyst is round in surface view, and dome-shaped in lateral view, since it is slightly flared at the base (Fig. 53). The base is hollow and acts as a support for the remainder of the cyst. The cyst proper averages 154 μ in diameter, and the base 184 μ; the total height averages 130 μ. The cyst wall is transparent but appears whitish to the unaided eye. The metacercaria retains its pigmentation, and the only structures even slightly visible are the oral sucker, the acetabulum, the eyes, the excretory pore, and the longitudinal striations in the cuticula described as being present in the contracted cercaria.

Under optimum conditions the metacercaria probably lives for several months as was pointed out by Krull (1934:176). To determine the longevity of the metacercaria 50 cercariae were allowed to encyst on the bottom of small dishes which were set aside and covered. Encystment occurred on June 5, 1934. On August 5, 1934, 56% were alive, and on September 5, 1934, 33% were alive. This experiment was carried no further but it is evident that under optimum natural conditions the metacercariae may live as long as 5 or 6 months. Krull kept them alive for 5 months under experimental conditions.

ADULT

Experimental Infestation

Development of Amphistome Parasites in the Final Host.—The information on this subject is very meager, consisting of chance observations and of only one concluded experimental problem. Looss (1892:166) observed that *Diplodiscus subclavatus* develops slowly in frogs during the winter months but he did not determine the time required for the worms to become mature. Beaver (1929:13) observed that a cercaria from *Planorbis trivolvis* was found to encyst on crayfish and frog larvae, and when fed to bullfrogs (*Rana catesbiana*) and snapper turtles (*Chelydra serpentina*) developed into a known species, *Allassostoma parvum*

Stunkard 1916. However, Beaver did not determine the time required for this species to become mature. Krull and Price (1932:34) determined experimentally that in lightly infested frogs *Diplodiscus temperatus* reaches maturity in 27 days but that in most hosts 2 or 3 months is the usual time required. Le Roux (1930:248) states that young forms of *C. cotylophorum* probably live in the duodenum of the final host 6 to 8 weeks before migrating into the rumen, where they become mature in another 6 to 8 weeks. Le Roux's statement was not supported by experimental evidence. Krull (1934:177) determined experimentally that *C. cotylophorum* matures in the latter part of the fourth month after

TABLE 14.—SHOWING RESULTS OF EXPERIMENTS IN WHICH CALVES WERE FED METACERCARIAE OF *Cotylophoron cotylophorum*

Host	Date of infestation	Date of examination	Number of metacercariae fed	Number of worms in duodenum	Number of worms in rumen
Calf I.....	Nov. 19, 1933	Jan. 4, 1934	150	0	6
Calf III.....	June 4, 1934	Jan. 25, 1934	300	199	4
	June 11, 1934		300		
	June 18, 1934		300		
Calf IV.....	June 4, 1934	July 25, 1934	300	19	34
	June 11, 1934		300		
	June 18, 1934		300		
	June 25, 1934		300		

infestation. His determination was based on the findings of eggs in the faeces of the host. Krull did not attempt to determine the age or size at which the parasite migrates from the duodenum to the rumen nor the size at which the parasite becomes mature after reaching the rumen.

Experimental Infestation of the Final Host of C. cotylophorum.—On June 28, 1933, two 7 or 8 months old calves were received at the animal pathology department of Louisiana State University. These animals were kept in a dry, well ventilated room when being used for experimental purposes but were allowed to graze in a small grassy plot at other times. No snails were present in this area so that there was no opportunity for the calves to become infested by *C. cotylophorum.*

These calves were obtained for experimental work in connection with this problem early in November, 1933. They were placed in the room previously mentioned on November 10, where they remained until the end of the experiment on January 4, 1934. The faeces of both calves were examined several times for the eggs of *C. cotylophorum* but were found to be negative. On November 19, 1933, they were separated by a

partition placed in the room, and Calf I was fed 150 metacercariae. Calf II was kept as a control (Table 14). Subsequently both animals were given the same type of feed and were given water from the same source.

TABLE 15.—SIZE (IN MILLIMETERS) OF *Cotylophoron cotylophorum* IN RUMEN OF EXPERIMENTAL CALVES

Note: In Calf I, infested 46 days, 6 worms were found in the rumen; in Calf III, infested 21 days, 4 worms; in Calf IV, infested 51 days, 34 worms (sizes of 20 are given here).

No.	Calf I	Calf III	Calf IV
1	3.25 x 1.71	2.06 x 0.63	1.95 x 0.78
2	3.37 x 2.13	2.10 x 0.63	1.95 x 0.91
3	3.55 x 2.15	2.29 x 0.73	2.08 x 0.72
4	3.60 x 2.20	2.95 x 0.84	2.08 x 0.85
5	3.67 x 2.08	.....	2.13 x 1.04
6	3.85 x 2.45	.....	2.28 x 0.85
7	.....	.....	2.31 x 0.91
8	.....	.....	2.34 x 0.80
9	.....	.....	2.34 x 0.96
10	.....	.....	2.34 x 1.04
11	.....	.....	2.47 x 0.85
12	.....	.....	2.54 x 0.78
13	.....	.....	2.60 x 0.78
14	.....	.....	2.60 x 0.85
15	.....	.....	2.60 x 0.91
16	.....	.....	2.60 x 0.98
17	.....	.....	2.62 x 0.85
18	.....	.....	2.70 x 0.83
19	.....	.....	2.73 x 0.88
20	.....	.....	2.86 x 0.99
Average	3.55 x 2.32	2.32 x 0.71	2.41 x 0.84

The faeces of both calves were examined at 2-day intervals after the twenty-first day of infestation. However, no eggs were found. Both animals were killed on January 4, 1934, after 46 days of infestation, in an attempt to establish the time of migration of the parasites. No parasites were found in Calf II, but 6 small mature specimens of *C. cotylophorum* were obtained from the rumen of Calf I. None was present in the duodenum. The parasites recovered varied in length from 3.25 to 3.85 mm and in width from 1.71 to 2.45 mm (Table 15). Only a few eggs were present in the uterus and only a few were deposited by the worms, although they were kept alive in physiological salt solution for 4 hours.

The presence of mature specimens in Calf I indicates that maturity is reached in less than 46 days, or that the worms were present as a result of natural infestation before the calf was confined and were depositing so few eggs that they were missed in the faecal examinations. The latter supposition is doubtless correct in view of the fact that Krull

(1934:177) determined in a series of experiments that this parasite becomes mature in approximately three and one-half months. Unfortunately, Krull did not determine the size at which this species becomes mature nor does he give the size of the worms recovered from the rumen of one of his experimental animals. However, Krull kindly loaned me one toto mount and one specimen serially sectioned, and a comparison of the present material to his is possible. Krull's specimens were approximately 6 months and 20 days old and the present material was 6 months and 7 days old, if we assume that Calf I became infested a short time before being confined. A comparison of size of the oral sucker, the esophageal thickening, the acetabulum, the testes, ovary, Mehlis' gland, and the

TABLE 16.—SHOWING NUMBER, SIZE (IN MILLIMETERS), AND DISTRIBUTION OF *Cotylophoron cotylophorum* IN THE DUODENUM OF EXPERIMENTAL CALF III

No.	1st foot (54 present)	2nd foot (40 present)	3rd foot (34 present)	4th foot (14 present)	5th foot (6 present)	6th foot (12 present)
1.....	1.22 x 0.72	0.99 x 0.36	1.35 x 1.05	2.08 x 0.78	1.61 x 0.39	1.56 x 0.80
2.....	1.50 x 0.95	1.43 x 0.85	1.43 x 0.75	2.26 x 0.99	1.90 x 1.06	1.97 x 1.06
3.....	1.69 x 0.93	1.45 x 1.05	1.43 x 0.85	2.39 x 0.80	2.50 x 0.93	2.26 x 0.85
4.....	1.60 x 0.80	1.69 x 0.96	1.71 x 1.04	2.60 x 0.78	2.54 x 1.04	2.54 x 0.91
5.....	1.90 x 0.95	1.85 x 1.10	2.13 x 1.04	2.67 x 1.01	2.75 x 1.01	2.60 x 1.04
6.....	2.00 x 0.85	2.13 x 1.04	2.30 x 1.15	2.73 x 0.93	3.09 x 1.04	2.75 x 0.96
7.....	2.00 x 1.05	2.25 x 0.75	2.50 x 1.25	2.75 x 1.01	.....	2.75 x 1.01
8.....	2.05 x 1.10	2.25 x 0.80	2.70 x 1.05	2.76 x 1.04	.....	2.75 x 1.14
9.....	2.15 x 1.05	2.55 x 0.95	2.75 x 1.15	2.86 x 1.06	.....	2.99 x 1.04
10.....	3.05 x 0.95	2.60 x 1.10	2.80 x 1.05	2.99 x 1.01	.....	3.09 x 1.04
Average..	1.94 x 0.94	1.95 x 0.90	2.11 x 0.96	2.61 x 0.94	2.23 x 0.90	2.63 x 0.99

genital sucker of Krull's sectioned material and similar structures in the smallest of the worms from Calf I demonstrates that the specimens are approximately the same age. The finding of no immature specimens in either the rumen or the duodenum indicates that none of the metacercariae fed to the calf developed.

This experiment was repeated in another attempt to determine the time of migration of parasites from the duodenum to the rumen. Two 4 months old calves which had not had access to infestation were obtained and kept under conditions similar to those of the first experiment. Calf III was fed 300 metacercariae on June 4, 1934, 300 on June 11, and 300 on June 18, making a total of 900 fed at 7-day intervals. Calf IV was fed a total of 1200 metacercariae at 7-day intervals from June 4 to June 25.

Calf III was examined on June 25, 1934, 21 days after the first infestation, and 203 immature specimens of *C. cotylophorum* were recovered. Of these, 199 were distributed in the anterior 6 feet of the

duodenum and 4 were found in the rumen. The other parts of the stomach were examined but no parasites were found. The smallest specimen from the duodenum measured 0.99 by 0.36 mm and the largest was 3.09 by 1.04 mm. There was no distinct grouping according to size so that it was impossible to determine the exact age of any one specimen. However, it may be safely assumed that the smallest worms were only 7 days old, whereas the largest were 21 days old. The large and small individuals were distributed irregularly in the duodenum so that it was again impossible to group them according to their distribution (Table 16). However, the largest number of small specimens was found in the upper 3 feet of the duodenum, indicating that the metacercariae probably excyst in this region. Most of the worms distributed posteriorly were much

TABLE 17.—SHOWING NUMBER, SIZE (IN MILLIMETERS), AND DISTRIBUTION OF *Cotylophoron cotylophorum* IN THE DUODENUM OF EXPERIMENTAL CALF IV

No.	1st foot	2nd foot	3rd foot	4th foot	5th foot
1	2.13 x 1.04	1.71 x 0.78	2.21 x 0.88	1.69 x 0.83	2.41 x 0.80
2	2.31 x 0.96	2.08 x 0.78	2.34 x 0.88	2.36 x 0.91	.....
3	2.34 x 0.91	2.62 x 0.72	2.34 x 0.91	.....	.....
4	2.36 x 1.04	.....	2.39 x 0.83	.....	.....
5	.....	.....	2.41 x 0.96	.....	.....
6	.....	.....	2.52 x 0.96	.....	.....
7	.....	.....	2.54 x 0.85	.....	.....
8	.....	.....	2.60 x 1.09	.....	.....
9	.....	.....	2.73 x 0.85	.....	.....
Average	2.28 x 0.99	1.60 x 0.76	2.44 x 0.91	0.02 x 0.87	2.41 x 0.80

larger than the smallest worms, indicating that the worms either migrate after becoming excysted anteriorly or that some metacercariae are carried farther before being liberated. The average size of the 199 specimens was 2.28 by 0.94 mm.

The 4 specimens found in the rumen of this host indicate that migration begins at the end of the first 3 weeks after infestation. These specimens varied from 2.06 to 2.95 mm in length and from 0.63 to 0.84 mm in width (Table 15). The average size of the 4 was 2.32 by 0.71 mm.

Calf IV was examined on July 25, 1934, 51 days after the first infestation and 30 days after the last infestation. Nineteen specimens were recovered from the first 5 feet of the duodenum, 34 from the rumen, and 1 from the pylorus (Table 17). Those from the duodenum were much more uniform in size than those from the duodenum of Calf III (Table 16). The smallest specimen measured 1.95 by 0.78 mm and the largest 2.86 by 0.99 mm. The average size of the 19 from the duodenum was 2.32 by 0.75 mm, while the average size of the 34 from

the rumen was 2.33 by 0.83 mm. The one specimen from the pylorus measured 2.43 by 0.81 mm.

The small number of parasites present in this host and their uniformity in size indicates that probably only one group of metacercariae

TABLE 18.—DATA ON SIZE (IN MILLIMETERS) OF *Cotylophoron cotylophorum* IN THE DUODENUM AND RUMEN OF NATURALLY INFESTED HOSTS

No.	Age of host: 6 months (16 present in duodenum) (7 present in rumen)		Age of host: 6 months (7 present in duodenum) (39 present in rumen)		Age of host: 2 years (none present in duodenum) (128 present in rumen)
	Duodenum	Rumen	Duodenum	Rumen (7 smallest)	Rumen (20 smallest)
1	1.04 x 0.41	2.54 x 1.17	0.96 x 0.83	2.44 x 1.19	1.82 x 1.22
2	1.22 x 0.52	2.62 x 1.30	1.35 x 0.78	2.60 x 1.30	1.87 x 1.08
3	1.97 x 0.80	2.63 x 1.04	1.43 x 0.85	2.62 x 1.56	1.92 x 1.04
4	2.00 x 0.91	2.70 x 0.98	1.56 x 0.91	2.71 x 1.56	1.92 x 1.17
5	2.10 x 0.96	2.99 x 1.22	1.87 x 1.06	2.83 x 1.53	2.00 x 1.06
6	2.15 x 0.85	3.12 x 1.30	2.08 x 0.98	3.12 x 1.45	2.00 x 1.17
7	2.21 x 0.65	3.30 x 1.01	2.39 x 0.78	3.12 x 1.58	2.02 x 1.17
8	2.23 x 0.78	.....	.....	.....	2.05 x 1.17
9	2.26 x 0.70	.....	.....	.....	2.08 x 1.04
10	2.26 x 0.72	.....	.....	.....	2.08 x 1.09
11	2.26 x 0.78	.....	.....	.....	2.08 x 1.11
12	2.34 x 0.78	.....	.....	.....	2.08 x 1.17
13	2.36 x 0.98	.....	.....	.....	2.08 x 1.17
14	2.52 x 0.98	.....	.....	.....	2.15 x 1.11
15	2.62 x 0.98	.....	.....	.....	2.21 x 1.17
16	2.70 x 0.91	.....	.....	.....	2.21 x 1.30
17	.....	.....	.....	.....	2.26 x 1.06
18	.....	.....	.....	.....	2.34 x 1.17
19	.....	.....	.....	.....	2.47 x 1.22
20	.....	.....	.....	.....	2.52 x 1.30
Average	2.15 x 0.72	2.86 x 1.15	1.66 x 0.88	2.78 x 1.45	2.10 x 1.14

fed to the calf produced the infestation. The average size of worms from the duodenum of Calf IV is only slightly greater than that of the worms from the duodenum of Calf III, which seems to indicate that it was the last feeding of metacercariae to Calf IV which produced the infestation. A comparison of the average size of the parasites from the duodenum and the rumen of Calf IV clearly indicates that the worms were migrating and that very little growth occurs during their passage through the other parts of the stomach. That this passage is rapid is indicated by the fact that only one specimen was found in other parts of the stomach. The variability of the size of the worms in the rumen combined with the fact that there is a decided overlapping of size with those in the duodenum indicates that the worms migrate singly and that the

migratory period for any group of worms of the same age may extend over several days. Thus in Calf III, only 4 of 203 worms had migrated at the end of 21 days, and in Calf IV, 34 of 54 had migrated at the end of 30 days.

The size at which the worms migrated from the duodenum to the rumen in the experimental animals can be correlated with findings in naturally infested hosts. Many naturally infested animals were examined which contained the parasites in both the duodenum and the rumen. Others were examined in which only the duodenum or rumen was infested. Three such cases are presented in Table 18. The host represented in the first column was infested by 128 specimens in the rumen, 15 of which were mature. The average size of 20 of the smallest specimens is 2.1 by 1.14 mm. These worms are slightly shorter but wider than those from the rumen of experimental animals.

The parasites shown in column 2 of Table 18 were collected from the duodenum and rumen of a 6 months old calf. Sixteen were found in the duodenum which had an average size of 2.15 by 0.79 mm. Only 7 were found in the rumen. These averaged 2.86 by 1.15 mm. The parasites shown in column 3 were collected from another 6 months old calf. In the duodenum of this calf there were 7 parasites which averaged 1.66 by 0.88 mm. There were 39 specimens in the rumen, the largest of which measured 5.33 by 1.82 mm. The average size of 7 of the smallest specimens was 2.78 by 1.45 mm.

In these three cases there is further evidence that the worms migrate from the duodenum into the rumen of the final host when considerably less than 3 mm in length. A graph made using the data on specimens from both experimentally and naturally infested animals demonstrates that the greater number of worms migrate at a size of 2.37 by 0.98 mm.

From the above data it is possible to conclude that the metacercariae become excysted in the duodenum where they develop for 3 to 5 weeks. Following this period they migrate to the rumen at an average size of 2.37 to 0.98 mm.

Development

The metacercariae excyst in the upper part of the duodenum but some may be carried as far posterior as 6 feet, as has been previously pointed out. The distribution of the various sizes found in the duodenum of experimental hosts indicates that some migration may occur within the limits in which they were found. The young forms are very active and migration for considerable distances is probably a matter of a very short time.

In the duodenum they are found attached to the mucosa by a power-

fully developed acetabulum, elongating and weaving their bodies from side to side. The worms are capable of moving rapidly from one position to another in the measuring worm manner. The color of the worm is reddish, which makes it very inconspicuous against the background of mucosa. To collect the worms, sections of the duodenum were placed in warm physiological salt solution. This increases their activity which makes them more easily seen. They were then scraped off and shaken free of all tissue from the duodenum.

The shape of the young worm is very much like that of the adult, being attenuated at the anterior end and widest in the testicular region. The dorsal surface is convex and the ventral surface is slightly concave. In cross section the body is nearly ovoid or round. The acetabulum is subterminal in living specimens, but when allowed to die unfixed the acetabulum opens posteriorly and the body becomes much flatter. The young worms are more active than the adults and are able to extend the body three times their contracted length while the adults are not capable of extending the body more than one and a half times their contracted length. Young individuals are also much more resistant than the adults. Some of the young specimens from the duodenum remain alive for 24 hours in cold physiological salt solution while older worms remain alive only 6 to 8 hours under similar conditions.

The age and size at which these parasites become mature was not determined experimentally, but by examining naturally infested animals a series of developmental stages varying from 1 to 11 mm in length was obtained. The smallest mature specimens measure 2.86 by 1.22 mm. Many are mature at a length of 3 mm. Krull (1934) has shown that these worms reach maturity in approximately three and one-half months, and the correctness of his findings has been pointed out previously (page 82). Since the worms migrate at an average size of 2.37 by 0.98 mm during the fourth and fifth weeks after infestation of the host and mature at the sizes given above it is evident that the rate of growth in the rumen is slow. This is shown also by the average size of the worms from the rumen of Calf I. Those worms which were 6 or 7 months old averaged only 3.55 by 2.32 mm.

The results obtained from the examination of a bull brought in to the animal pathology department of Louisiana State University are also of value in demonstrating the slow growth rate of these forms. This bull was brought in for observation on August 10, 1933, and was kept, as were the experimental calves, with no chance of becoming infested with *C. cotylophorum*. Upon examination on June 12, 1934, 26 specimens of *C. cotylophorum* were found in the rumen. The smallest of these worms measured 5.2 by 2.1 mm and the largest 7.0 by 2.75 mm. The average

size of the 26 was 5.6 by 2.34 mm. This bull had been confined slightly more than 10 months so that the smallest of the specimens must necessarily have been somewhat over 10 months of age. The largest specimens found from other naturally infested hosts never exceeded 11 by 3 mm. Specimens of this size were probably well over one year in age and represent the maximum size attained by the parasites in this host. No information other than the above was obtained concerning the longevity of these forms.

The adult of this species has been fully described by Fischoeder (1901, 1903), Stiles and Goldberger (1910), Maplestone (1923), Bennett (1928), and Stunkard (1929) so that no detailed descriptions of structures will be given here. Descriptions of the development of structures of diagnostic importance and of structures which have not been described for this parasite are included.

Digestive Tract.—In the smallest individuals the digestive tract is identical in appearance to that of the largest worms. The caeca are in the same position in both, that is, in the dorso-lateral part of the body and terminate dorsal to the acetabulum. The posterior ends may be curved ventrally anterior to the acetabulum, but such variations are of no importance as pointed out by Maplestone (1923) and can be explained on the basis of differences in the degree of contraction.

The oral sucker changes consist of an increase in size only. Its growth, however, is not proportionate to that of the body. Its length in 1 mm worms as compared with the body length is in the ratio of about 1:5; in 2 to 3 mm worms the ratio is 1:6 or 1:7; and in 4 to 5 mm worms the ratio is 1:6. The oral sucker attains its maximum size in worms of 6 to 7 mm and the ratio is about 1:9. In a well extended specimen measuring 6 by 2.5 mm the oral sucker is 0.74 mm long, 0.58 mm wide and 0.46 mm dorso-ventrally. The worms may reach a size of 11 by 3 mm but there is no further increase in the size of the sucker.

The esophagus is the only structure of the digestive tract which possesses characteristics of specific importance. These are its length and the gradual increase in thickness of its walls from its anterior to its posterior end. The length of the esophagus is subject to considerable variation because of contraction but it increases in length as the worms develop, reaching its maximum length in worms of 6 to 7 mm in length as does the oral sucker. In the smallest worms obtained the esophagus is about 0.3 mm long and it increases steadily as the worms grow, but it never exceeds 0.9 mm in length. It bifurcates to form the intestinal caeca in the region of the genital pore in worms under 7 mm long (Figs. 81, 82, 83). However, the worms reach 11 mm in length and as a result the genital pore becomes distinctly post-bifurcal in position.

Stiles and Goldberger (1910:72) state that the genital pore of *C. indicum* is decidedly post-bifurcal and designate this as one of the differences between *C. indicum* and *C. cotylophorum*. Maplestone (1923:152) has pointed out that this characteristic is too variable to be of diagnostic importance for these two species, and the present findings support his contention.

The muscular thickening of the esophagus in *C. cotylophorum* is evident in the cercaria and becomes more evident as the worm develops in the final host. In the small worms the muscle wall at the proximal end of the esophagus is about 5 μ thick and at its distal end is 15 μ thick, while the diameter of the esophagus at the proximal end including the esophageal glands is 37 μ and at the distal end is 85 μ. The ratio of these measurements is found to vary from 1:2 in young worms to 1:4 in old worms. The thickness of the muscle wall at the proximal end does not exceed 20 μ, and it does not exceed 60 μ at the distal end. In the largest of the worms the total diameter of the proximal end does not exceed 0.17 mm and does not exceed 0.32 at the distal end. All of these measurements are subject to considerable variation but there is a normal increase in the size of the esophagus concurrent with growth. However, growth of the esophagus stops when the worms reach a size of 6 or 7 mm.

The difference in the thickness of walls of the esophagus at the two ends is very evident at all ages (Figs. 70-73), and the appearance of this structure is very similar to that in *C. cotylophorum* as described by Fischoeder (1903:547) and Maplestone (1923-152).

Maplestone (p. 152) attempts to show that the esophagus of *C. indicum* and *C. cotylophorum* are identical, but I cannot agree with him. As stated, the esophageal thickening in the present material is very evident at all ages and sizes of worms. Stiles' and Goldberger's material possessed no such thickening, although their figure (1910:fig. 45, p. 66) shows that the walls of the esophagus are thick. The apparent muscular thickening shown in their figure is due to an increase in the lumen rather than to an increase of thickness of the walls. I have been able to determine this point from a sectioned specimen of a worm identified as *C. indicum* by these writers. The thickness of the walls of the esophagus in this material does not exceed 10 μ at its proximal end and does not exceed 20 μ at its distal end. The diameters of the two ends are 0.105 and 0.195 mm respectively. The above worm, which was not mature, measured 5.16 by 1.68 mm. These differences are too great to be the result of normal variation, as determined by a comparison with the present material.

Development of Sex Organs.—The primordia of the genital organs

are present in the cercaria, as previously described. In the smallest worms recovered from the final host the ovary, Mehlis' gland, and testes were differentiated (Fig. 77). The ovary and Mehlis' gland are represented by two small masses of cells located near the center of the body above the anterior margin of the acetabulum. From Mehlis' gland a cord of cells, in which there is no lumen, passes ventrally over the anterior margin of the acetabulum to the ventral region of the body. It turns forward and continues anteriorly near the ventral surface to reach a mass of cells located above the position of the future genital pore. The testes are located laterally, one on each side of the cord immediately anterior to the acetabulum. Their method of formation was not determined, but their position suggests that they are set off from the ventral side of the mass of cells located anterior and dorsal to the acetabulum in the cercaria. The ovary and Mehlis' gland doubtless are formed from the dorsal part of this mass. There is no indication of a lumen in the cord of cells at any place. The testes are surrounded by a thin membrane but no vasa efferentia were observed. The genital pore is not yet formed, and the only indication of a genital sucker is a slight thickening of the body wall and a mass of deeply-staining cells which are probably cells of the prostate gland.

The degree of development described above is reached in worms of approximately 1.0 by 0.65 mm. In individuals of this size the ovary measures 0.045 by 0.03 mm, Mehlis' gland 0.037 by 0.022 mm, the testes 0.065 by 0.045 mm, the diameter of the cord of cells 0.045 mm, and the mass of cells surrounding the genital pore 0.097 by 0.032 mm.

The vasa efferentia, the vas deferens, the uterus, and a genital pore were clearly distinguished in an individual 1.17 by 0.8 mm. The uterus follows a course from Mehlis' gland to the ventral side of the body similar to that of the cord of cells described in the above form and is doubtless formed from it. Here it turns dorsally and anteriorly until it reaches the center of the body. It passes forward for a short distance and then turns ventrally. Near the ventral surface it turns forward to the genital pore. It joins the vas deferens, and the common duct thus formed, the ductus hermaphroditicus, opens to the outside (Fig. 63).

In this same specimen the testes have taken the tandem arrangement characteristic of the mature worm. The vasa efferentia are conspicuous and are easily traced. The one from the posterior testis passes anteriorly and dorsally until it reaches the mesial side of the right caecum where it turns forward. The vas efferens from the anterior testis passes forward mesial to the left caecum. The two unite in the center of the body a short distance posterior to the genital pore and immediately anterior to the descending uterus. The vas deferens thus formed coils ventrally and

anteriorly to the genital pore. It is located dorsal to the uterus and unites with it to form the ductus hermaphroditicus.

The position of the vasa efferentia and vas deferens indicates that these structures are formed from the dorsal part of the cord of cells described in the smaller individuals. The ventral position of the uterus near the genital pore indicates that it is derived from the ventral part of the cord. The descent of the uterus posterior to the loop formed by the union of the vasa efferentia supports this conclusion also.

The genital sucker becomes more evident in specimens of this size, although as yet there are no definitely differentiated muscles in it. It consists of a compact mass of cells surrounding the terminations of the vas deferens, the uterus, and the ductus hermaphroditicus. This mass of cells is approximately 0.12 mm long, 0.108 mm wide, and 0.072 mm thick (Fig. 63).

All of the male and female genital structures, with the exception of the vitellaria, are formed before the worms migrate from the duodenum to the rumen.

Following the stage just described the other structures characteristic of the genitalia of the mature worm are rapidly developed, being present in worms 2.5 mm long and less than 3 weeks old (Fig. 76). The vas deferens is differentiated into several distinct regions: a seminal vesicle, a pars musculosa, the prostate gland, and the ductus ejaculatorius, which joins the ductus hermaphroditicus. The ductus hermaphroditicus passes through the center of a minute hermaphroditic papilla which opens into a small genital atrium in the genital papilla. The copulatory structures and the terminations of the male and female ducts are enclosed in the genital sucker.

Laurer's canal is well developed in individuals of this size also. It passes from the oviduct dorsally and laterally to open to the exterior behind the excretory pore and to the left of the median line. The only other development in the female system is the formation of the metraterm.

There is no recognizable change in the worms immediately following their migration into the rumen, which is a second indication that the time required for migration is very short. The fact that only one worm was found in other parts of the stomach other than the rumen has been considered above as an indication that the worms migrate from the duodenum to the rumen rather quickly.

No worms of known age were studied from the time of migration but many specimens representing all sizes and stages of development were secured from naturally infested hosts. There is very little increase in size before the worms reach sexual maturity and this increase is in

diameter. The smallest mature worm collected measured 2.86 by 1.43 mm. The most conspicuous changes in the small mature worms as compared to the immature ones are the increase in size of the genital organs and the development of the vitellaria (cf. Figs. 76 and 79). In the smallest of the mature worms the vitellaria are very sparsely developed in the lateral regions of the body and extend from the esophagus to the acetabulum. The testes in immature worms at the time of migration are round and smooth and measure only 0.1 mm in diameter. In the smallest of the mature worms the testes are 0.36 by 0.26 mm; they extend dorsoventrally for a distance of 0.48 mm and are distinctly lobed. There is an increase in the size of the ovary from 0.075 mm to 0.196 mm in diameter. The uterus in both the immature and small mature worms is straight but its diameter increases from 0.032 mm in the immature to 0.081 mm in the mature worms. The vasa efferentia remain unchanged but the vas deferens becomes greatly coiled. The genital sucker and copulatory structures are also much more conspicuous in the small mature worms.

The genital sucker in worms which have just migrated to the rumen measures 0.17 mm in diameter and the muscle mass is about 0.14 mm thick (Fig. 76). In small mature worms this structure measures 0.4 mm in diameter and is 0.22 mm thick.

The only marked change which occurs after sexual maturity is attained is the rapid development of the vitellaria. In worms only 3.5 mm in length they have reached a state of development comparable to that in the largest worms (Fig. 80). They extend in closely grouped follicular masses from the oral sucker to the acetabulum, principally in the extra-caecal zones but approach the median line both dorsally and ventrally. The uterus becomes more coiled as larger numbers of eggs are produced and fills all available space between the acetabulum and the posterior testis. It is coiled transversely dorsal to the testes, and more coils are formed in the ventral region of the body anterior to the anterior testis.

Stunkard (1929:244) found that *C. cotylophorum* reached maturity at a much smaller size in calves than in antelopes, but he could not explain the incongruity. He did not give the size of the mature worms from the calves but specimens as long as 6 mm from the antelopes were not mature. These findings clearly indicate that these parasites mature at a much smaller size in some hosts than in others.

The genital organs are much larger in fully grown worms than in those just reaching maturity (cf. Figs. 79 and 81). The testes are located in tandem arrangement near the center of the body and occupy approximately two-fifths of the body length. The seminal vesicle is

greatly expanded and coiled; the pars musculosa is thick, muscular-walled, and coiled; the pars prostatica located directly above the genital pore is straight and measures about 0.20 by 0.22 mm. Its lumen is large but narrows abruptly as it becomes continuous with the ductus ejaculatorius. The ductus ejaculatorius is about 0.19 mm long and opens into the ductus hermaphroditicus. The hermaphroditic papilla varies in length with its state of contraction but its length is about 0.1 mm. The genital papilla encloses a small atrium into which the protrusible hermaphroditic papilla projects. The walls of the genital papilla are very muscular and are about 50 μ thick. The genital papilla when protruded measures about 0.19 by 0.14 mm and projects into the cavity of the genital sucker (Fig. 74).

The ovary and Mehlis' gland in fully grown worms are located above the anterior margin of the acetabulum. The uterus is crowded with eggs and is coiled anterior to the acetabulum, dorsal to the testes, and in the ventral region of the body anterior to the anterior testis. The muscular metraterm joins the ductus hermaphroditicus. The union of the male and female ducts forms a distinct but small vesicle at the inner end of the ductus hermaphroditicus.

The genital sucker consists of a muscular mass which encloses the terminal ends of the male ducts and the copulatory apparatus. This structure increases in size as the worms develop but does not exceed 0.7 mm in diameter in any of the present material. Its walls are approximately 0.3 mm thick. The cavity of the sucker is considered as a genital atrium by Fischoeder (1903) and Maplestone (1923). The genital pore is the opening of the sucker while the pore of the ductus hermaphroditicus is the porus hermaphroditicus.

When the worms are emitting eggs or sperm the genital papilla can be protruded for a short distance beyond the edge of the genital sucker. At the same time rapid contractions and relaxations of the muscles of the genital sucker occur.

The genital atrium, the genital papilla, and the genital sucker are subject to considerable change in shape and size. The hermaphroditic papilla is also subject to great changes in size and appearance. However, in the present material after their development was completed these structures were evident in all ages and sizes of worms, and in all states of contraction (Figs. 68, 69, 74, 75).

Maplestone (1923:153-155, figs. 8, 9) describes in detail the extreme variability in the appearance of the genital sucker and copulatory apparatus of C. *cotylophorum* and concludes that these structures are of no diagnostic value. His figures (Figs. 8, A1, A2, B) represent the appearance of the genital sucker and copulatory apparatus of *C.*

cotylophorum as described by Fischoeder (1903:548, fig. 38) and of *C. indicum* as described by Stiles and Goldberger (1910:69, fig. 48). As a result of his observations on these structures and on the variability of the esophagus of *C. cotylophorum* he considers *C. indicum* as a synonym of *C. cotylophorum.*

Fukui (1929:319) agrees with Maplestone and designates *C. indicum* as a synonym of *C. cotylophorum.*

As originally described by Fischoeder the genital sucker of *C. cotylophorum* is much larger and much more distinctly set off from the body parenchyma than in Maplestone's or the present material (Fig. 67). Fischoeder does not describe a genital papilla in *C. cotylophorum* and the male and female ducts remain separate in the hermaphroditic papilla.

The genital sucker of *C. indicum* as described by Stiles and Goldberger is also distinctly set off from the body parenchyma and they do not describe a genital papilla as being present. In the present study some of the original material of Stiles and Goldberger was studied and no genital papilla was found. There is also a distinct difference in the appearance of the genital sucker of *C. indicum* and that of *C. cotylophorum* as described and figured by Maplestone (1923:156, fig. 9) and as described in this paper.

Stunkard (1923:138) believes that Maplestone's conclusions as to the importance of these structures are erroneous. I am of the same opinion, and in view of the decided differences in the appearance of the genital sucker, the copulatory apparatus, and esophagus of *C. indicum* as compared to *C. cotylophorum* I cannot consider these two species as synonymous.

The present specimens of *C. cotylophorum* agree in every detail with Maplestone's description of this species with the exception of the extreme variability of the copulatory apparatus as pointed out above, with Stunkard's description (1929:244-251), and with specimens loaned me by Krull. However, I believe that the differences between the genital sucker and copulatory apparatus of Fischoeder's material and the present material are of diagnostic importance. Since these differences are the only ones which have been observed I am hesitant in considering these differences as being of specific value in view of Maplestone's and Fukui's findings.

Excretory System.—The arrangement of the excretory system in the young and mature specimens is very similar to that of the cercaria (Fig. 60). The details of the system were not studied. Only living immature specimens under pressure were studied. It was possible in this way to determine the course and extent of the larger ducts and the position of the bladder.

The bladder is an elongate structure located dorsally in the posterior region of the body, extending from near the posterior margin of the acetabulum to slightly past the anterior margin. It opens to the exterior through a narrow short muscular duct lined with cuticula continuous with that of the body surface. It may pass directly to the surface from the middle or anterior end of the bladder or may extend forward from it (Figs. 77, 78). In young specimens the former condition is more often found, and it is only in the more fully developed and very extended individuals that the duct opens very far in front of the bladder. The pore is located in the medial dorsal line, usually directly above the posterior margin of the posterior testis in mature specimens, but it may be as far forward as the middle of the anterior testis, or as far posterior as the anterior margin of the acetabulum. Its position relative to these organs depends entirely on the age of the individual and its state of contraction. However, the position of the pore may be considered as prevesicular, being dorsal to the bladder only in immature or contracted mature specimens. Maplestone (1923:157, text-fig. 11) has described and shown similar conditions in specimens of *C. cotylophorum* of different ages. I did not observe the pore to be post-vesicular in any of my material as Maplestone has figured it in a very young specimen. Fukui (1929:275) has described similar variations in *Paramphistomum explanatum, P. cervi,* and *P. orthocoelium.* He states that the pore is very variable in position and cannot be used for exact diagnostic purpose, but that it is roughly definite for species.

The main excretory canals are located the same as in the cercaria. From their union with the posterio-lateral angle of the bladder on each side they pass outward and forward. Immediately posterior to the middle of the body length these canals bend mesially and a cross connection passes across the middle line and sends off a forward diverticulum. The main canals then curve outward and forward. The diverticulum present just posterior to the eye in the cercaria is present in these older worms, and in them it receives a small duct which in turn receives branches from the esophageal region. The main canals continue forward from this diverticulum until they reach the posterior margin of the oral sucker. Here they turn abuptly on themselves and pass posteriorly in the lateral regions of the body. These posterior extensions could not be traced to their terminations but doubtless they extend as far back as the acetabulum, as they do in the cercaria. A small duct on each side extends forward from the turning point of the main canals which drains the anterior region of the body. Numerous small ducts which are symmetrically located empty into the larger canals throughout their course.

The anterior diverticulum from the cross connection becomes greatly enlarged and it also receives small ducts from the anterior dorsal region of the body.

A detailed description of the excretory system was made from preserved material by Bennett (1928:22-23).

The excretory system of this material is very similar to that of Gastrothylax and Paramphistomum as described by Fukui (1929:272). This type of system he designates as Type A and calls it H-shaped.

Specific Description of *Cotylophoron cotylophorum*

The following specific description of *C. cotylophorum* is based entirely on the characteristics of the material used in the present study.

Body of mature worm 3 to 11 mm long by 1.15 to 3 mm wide; conical in form, greatest width in testicular region; tapers to bluntly pointed anterior end, posterior end broadly rounded; dorsal surface convex longitudinally and transversely, ventral surface concave longitudinally, convex transversely; oval to round in cross section. Surface without spines or papillae. Genital pore bifurcal or slightly post-bifurcal, at junction of first and second body thirds, surrounded by genital sucker 0.4 to 0.7 mm in diameter which forms a distinct projection in the median ventral line. Acetabulum at posterior end, distinctly subterminal 0.75 to 1.36 mm in diameter. Mouth at blunt anterior extremity; oral sucker pyriform in sagittal section; 0.52 mm long, 0.45 mm wide and 0.39 mm in dorso-ventral diameter in small mature worms, its maximum in fully grown individuals 0.74 mm long, 0.58 mm wide and 0.45 mm in dorso-ventral diameter; esophagus slightly longer than oral sucker; its walls increase in thickness posteriorly, ratio of thickness of anterior wall to posterior wall 1.3; caeca arise from dorso-lateral aspects of end of esophagus, terminate in acetabular zone. Excretory pore in median dorsal line about at junction of median and posterior body thirds; excretory bladder extends posteriorly from pore above acetabulum; lateral excretory tubes extend from ventral and postero-lateral margin of bladder to oral sucker, turn sharply posterior to acetabulum; cross connection between lateral ducts in dorsal region and near middle of body length, median diverticulum extends forward for short distance from cross connection, lateral diverticulum from each lateral duct a short distance posterior to oral sucker.

Testes large, lobate, about size of oral sucker in young mature worms, larger than acetabulum in old specimens, in median line, tandem arrangement; union of vasa efferentia slightly anterior to anterior testis; vas deferens coiled; its vesicula seminalis coiled, expanded; pars musculosa coiled, narrow; pars prostatica straight, located directly above genital

sucker; ductus ejaculatorius short, unites with metraterm to form ductus hermaphroditicus; hermaphroditic papilla short, protrusible, arises from the vertex of a conspicuous genital papilla, almost filling the cavity of the papilla; genital papilla in turn surrounded by the genital sucker.

Ovary and Mehlis' gland above anterior margin of acetabulum; Laurer's canal passes over excretory bladder, opens posterior to excretory pore and left of median dorsal line; uterus coiled anterior to acetabulum, passes anteriorly dorsal to testis, descends vertically over the anterior margin of anterior testis, anteriorly again ventral to vas deferens, enters genital sucker; and metraterm unites with ductus ejaculatorius.

SUMMARY AND CONCLUSIONS

Cotylophoron cotylophorum is a widely distributed parasite of ruminants but has not been previously reported from the mainland of North America.

The time required for the miracidium to develop varies directly with temperature. In the present experiments eggs kept at room temperatures hatched in 11 to 29 days.

The structures of the miracidium develop in sequence and are recognizable before hatching occurs.

The miracidium is similar to other amphistome miracidia. A study of the descriptions of 18 different species of miracidia indicates that the number and arrangement of ciliated epidermal cells is of taxonomic value.

The snails *Fossaria parva* and *F. modicella* are capable of serving as the intermediate hosts of *C. cotylophorum*. The former is the natural host of this parasite in Louisiana.

The miracidium penetrates the mantle, head, and foot of the snail, loses its ciliated epidermal cells, and transforms into a sporocyst.

The sporocyst develops rapidly and produces 9 rediae.

The rediae are born at an average size of 0.188 by 0.056 mm and migrate into the liver and ovo-testis where their development is complete. Each redia produces approximately 25 cercariae.

Mother rediae may occur in the life cycle but were observed in only one instance.

Cercariae are born in an undeveloped condition and continue their development in the liver and ovo-testis.

The time required for the development of the sporocyst, redia, and cercaria varies directly with temperature. Infested snails kept under natural temperature conditions shed cercariae in 30 to 91 days.

The cercariae encyst on vegetation and the metacercaria lives for over 3 months.

The metacercariae become excysted in the duodenum of the final host. Migration from the duodenum to the rumen begins in 21 days at an average size of 2.37 by 0.98 mm and may continue over a period of about 14 days. The worms do not migrate at the same age or size.

The worms become mature after reaching the rumen at an age of about three and a half months, at a size of approximately 3.0 by 1.15 mm. They reach their maximum size in about one year.

The time required to complete the life cycle of *C. cotylophorum* varies from about 5 to 8 months.

C. indicum is not a synonym of *C. cotylophorum.*

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida with the exception of Fig. 80 which is a graphic reconstruction.

Abbreviations Used

a.....acetabulum
an.....anterior nerve
ap.....apical papilla
bm.....basement membrane
bp.....birth pore
br.....brain
c.....cuticula
ca.....caecum
ca p.....primordium of caecum
cc.....central cavity
ceb.....caudal excretory bladder
ced.....caudal excretory duct
cep.....caudal excretory pore
cer.....cercaria
cg.....cerebral ganglion
cm.....circular muscles
com.....commissure
cu c.....cuticular cell
cy c.....cystogenous cell
cyg.....cystogenous granule
de.....ductus ejaculatorius
dh.....ductus hermaphroditicus
eb.....excretory bladder
ec.....epithelial cell
ed.....excretory duct
em.....embryo
en.....eye nerve
ep_1.....epidermal cell, row 1
ep_2.....epidermal cell, row 2
ep_3.....epidermal cell, row 3
ep_4.....epidermal cell, row 4
epn_1.....epidermal cell nucleus, row 1
epn_2.....epidermal cell nucleus, row 2
epn_3.....epidermal cell nucleus, row 3
epn_4.....epidermal cell nucleus, row 4
es.....esophagus
es'.....esophageal cell
et.....excretory tubule
exp.....excretory pore
ey.....eye
fc.....flame cell
g.....gut
ga.....genital atrium
gb.....germ ball
gc.....germ cell
gn.....gut nucleus
gp.....genital pore
g pa.....genital papilla
gs.....genital sucker
gt.....germinal tissue
hp.....hermaphroditic papilla
i.....intestine
La c.....Laurer's canal
lc.....lens cell
le.....lateral evagination
led.....lateral excretory duct
lm.....longitudinal muscle
m.....metraterm
me.....median evagination
Mg.....Mehlis' gland
n.....nerve
nc.....nerve cell nucleus
nf.....nerve fiber
nu.....nucleus
op.....oral plug
os.....oral sucker
ov.....ovary
p.....plug
pe.....primitive epithelium
pg.....penetration gland
pgd.....penetration gland duct
ph.....pharynx
ph c.....pharnygeal cuticular cell
pi.....pigment
pm.....pars musculosa
pp.....pars prostatica
pn.....posterior nerve
sg.....salivary gland
sn.....subepithelial nucleus
sp.....sensory papilla
st.....sporocyst tissue
sv.....seminal vesicle
t.....tail
te.....testis
u.....uterus
v.....vitellaria
vd.....vas deferens
ve.....vas efferens

PLATE I

FIGS. 1-11.—Developing miracidia. Scale 0.05 mm.

FIG. 12.—Four-cell stage in development of the miracidium. Scale 0.03 mm.

FIG. 13.—Flame cell of miracidium. Scale 0.01 mm.

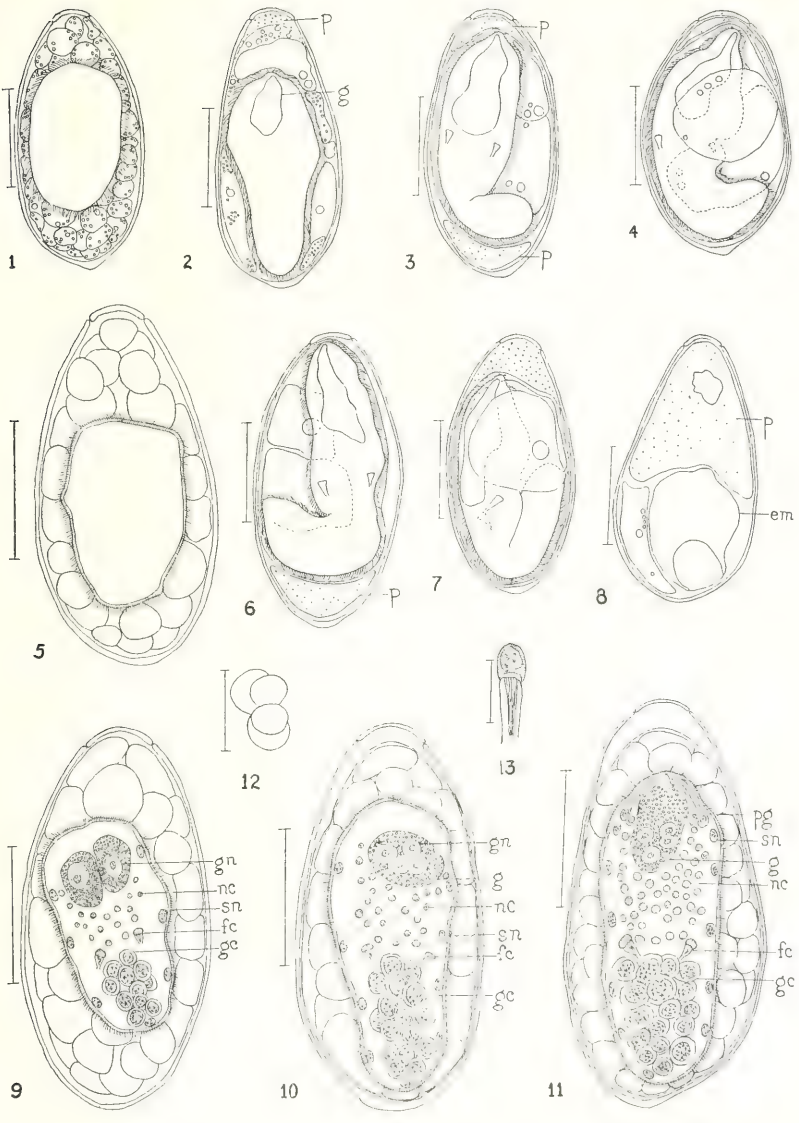
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PLATE I

PLATE II

FIGS. 14-17.—Mature miracidia. Scale 0.05 mm.

FIGS. 18-21.—Cross sections of miracidia. Scale 0.02 mm.

FIG. 22.—Frontal section of anterior end of miracidium. Scale 0.02 mm.

FIG. 23.—Frontal section of miracidium. Scale 0.02 mm.

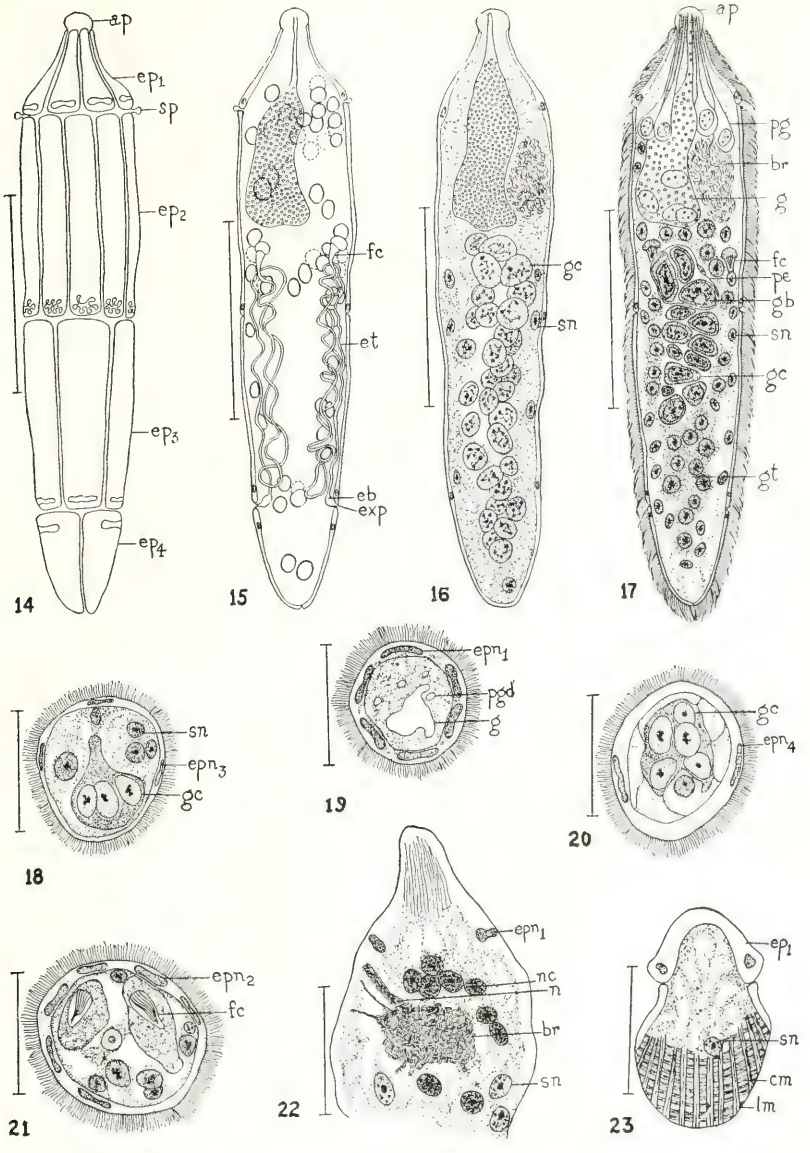
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PLATE II

PLATE III

FIG. 24.—Miracidium in lymph duct of snail. Scale 0.03 mm.

FIG. 25.—Five-day sporocyst. Scale 0.05 mm.

FIG. 26.—Mature sporocyst. Scale 0.1 mm.

FIG. 27.—Germinal and epithelial cells in body wall of sporocyst. Scale 0.01 mm.

FIG. 28.—Twenty-four-hour sporocyst. Scale 0.03 mm.

FIG. 29.—Twelve-hour sporocyst. Scale 0.02 mm.

FIG. 30.—Longitudinal section of sporocyst. Scale 0.1 mm.

FIG. 31.—Longitudinal section of forty-eight-hour sporocyst. Scale 0.03 mm.

FIG. 32.—Body wall of sporocyst. Scale 0.05 mm.

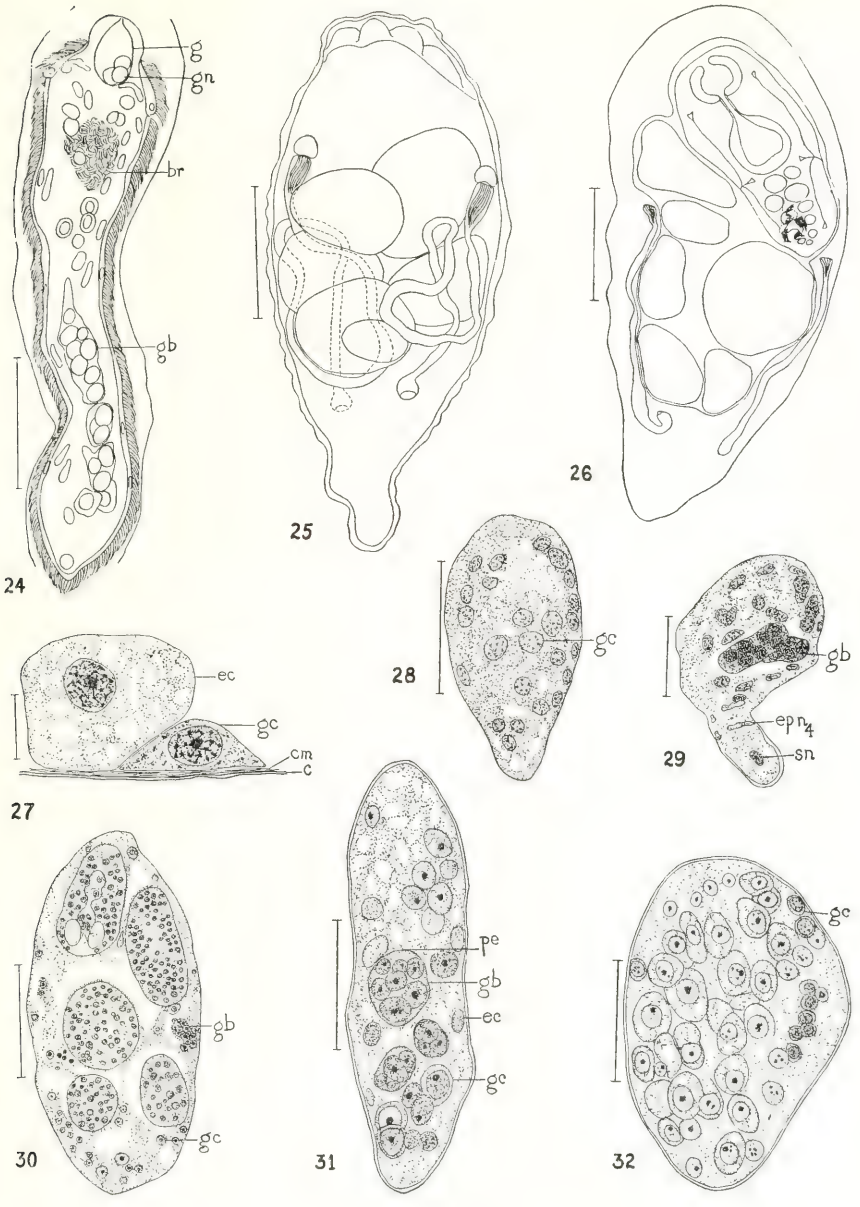
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PLATE III

PLATE IV

FIG. 33.—Mature redia. Scale 0.3 mm.

FIG. 34.—Frontal section of redia showing salivary glands. Scale 0.05 mm.

FIG. 35.—Mature sporocyst. Scale 0.1 mm.

FIG. 36.—Redia about to escape from sporocyst. Scale 0.05 mm.

FIG. 37.—Frontal section of redia showing brain. Scale 0.05 mm.

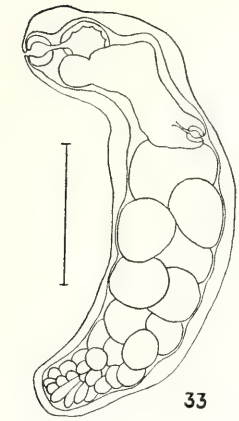
FIG. 38.—Sagittal section of anterior end of redia. Scale 0.1 mm.

FIG. 39.—Posterior end of redia showing germ cells and developing cercariae. Scale 0.1 mm.

FIG. 40.—Redia in pocket of sporocyst tissue. Scale 0.3 mm.

FIG. 41.—Posterior end of redia showing exhaustion of germ cells. Scale 0.1 mm.

FIG. 42.—Longitudinal section of immature redia. Scale 0.03 mm.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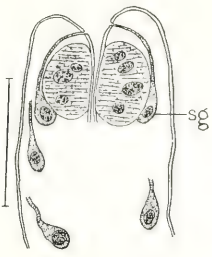

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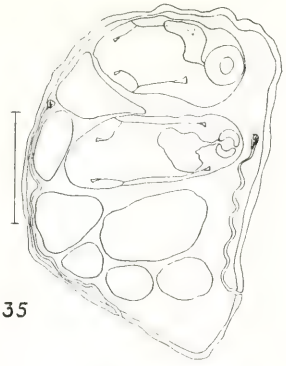
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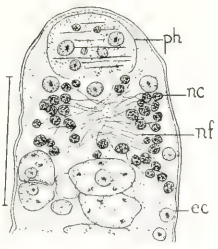

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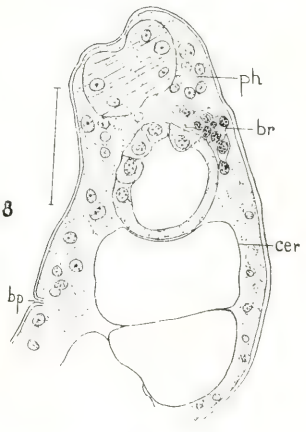

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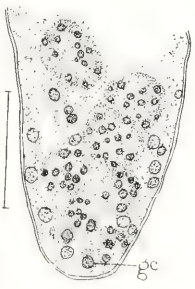

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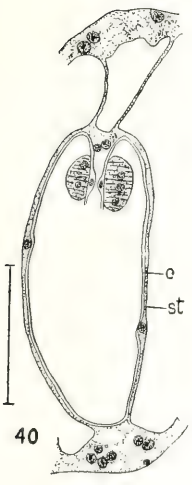

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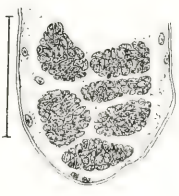
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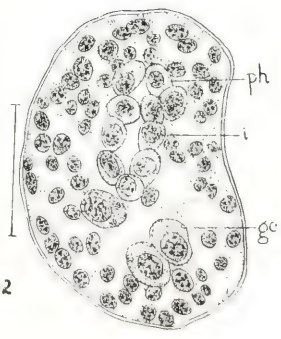

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PLATE IV

PLATE V

FIGS. 43, 44.—Longitudinal section of anterior end of immature redia. Scale 0.03 mm.

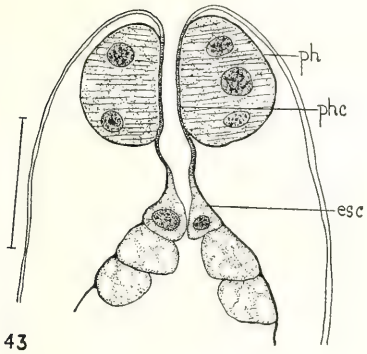
FIG. 45.—Lateral view of posterior end of cercaria. Scale 0.05 mm.

FIG. 46.—Mature redia. Scale 0.2 mm.

FIG. 47.—Oral sucker and esophagus of mature cercaria, sagittal section. Scale 0.02 mm.

FIG. 48.—Mature redia. Scale 0.2 mm.

FIG. 49.—Longitudinal section of anterior end of immature redia. Scale 0.03 mm.



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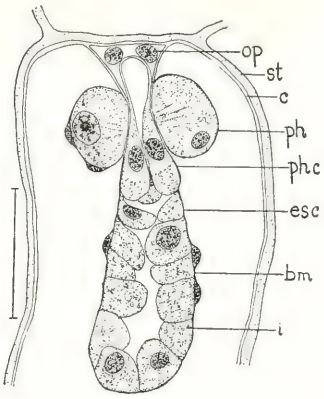

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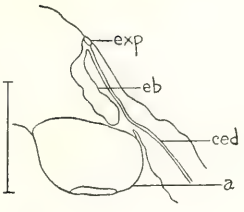

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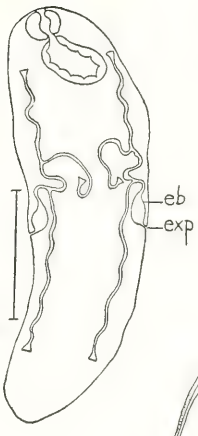

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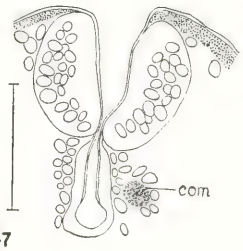

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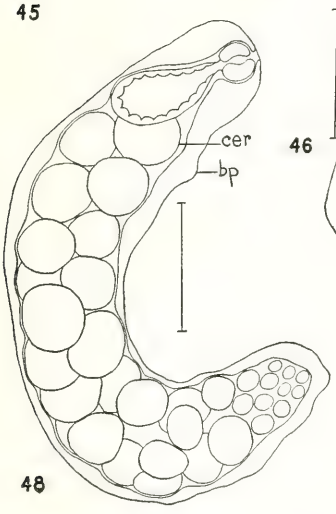

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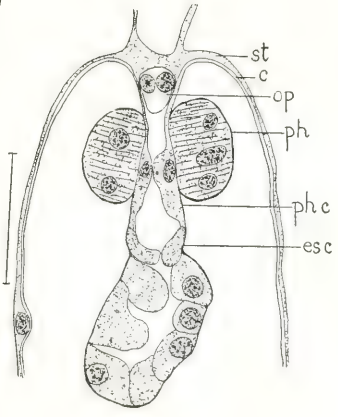

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PLATE V

PLATE VI

FIG. 50.—Immature cercaria, dorsal view. Scale 0.05 mm.

FIG. 51.—Mature cercaria, ventral view. Scale 0.1 mm.

FIG. 52.—Immature cercaria, dorsal view. Scale 0.05 mm.

FIG. 53.—Metacercaria, lateral view. Scale 0.01 mm.

FIG. 54.—Cross section of tail of mature cercaria. Scale 0.04 mm.

FIG. 55.—Frontal section of immature cercaria. Scale 0.05 mm.

FIG. 56.—Immature cercaria, dorsal view. Scale 0.05 mm.

FIG. 57.—Immature cercaria, ventral view. Scale 0.05 mm.

FIG. 58.—Mature cercaria, sagittal section. Scale 0.05 mm.

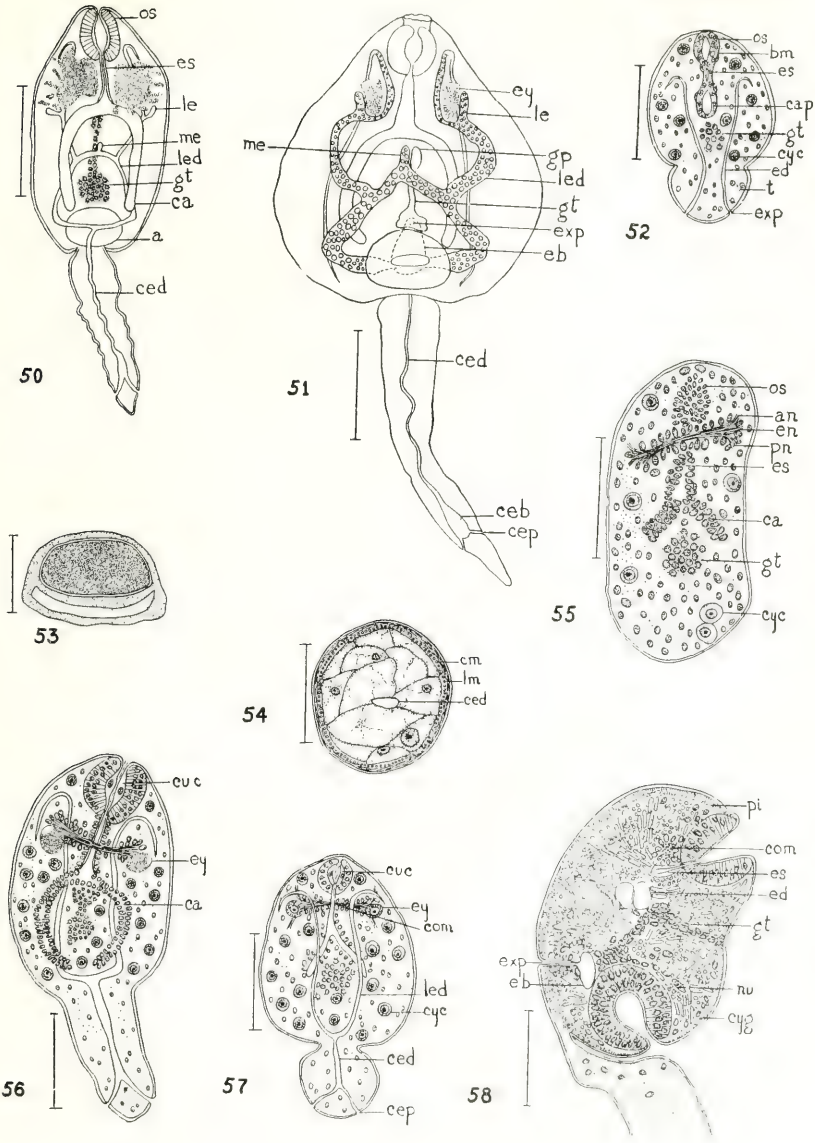
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PLATE VI

PLATE VII

FIG. 59.—Immature cercaria, dorsal view showing pigment. Scale 0.1 mm.

FIG. 60.—Excretory system, immature specimen. Scale 0.2 mm.

FIG. 61.—Immature cercaria, lateral view showing development of pigment. Scale 0.1 mm.

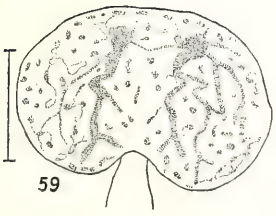
FIG. 62.—Section of developing eye. Scale 0.01 mm.

FIG. 63.—Cross section through genital sucker of a worm 1.17 x 0.84 mm. Scale 0.1 mm.

FIGS. 64, 65.—Sections of developing eye. Scale 0.02 mm.

FIG. 66.—Anterior end of mature cercaria, sagittal section. Scale 0.04 mm.

FIG. 67.—Cross section through genital sucker of a worm 3.65 x 2.45 mm. Scale 0.5 mm.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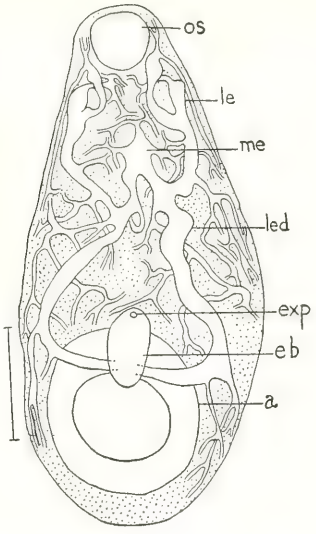
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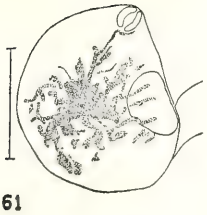
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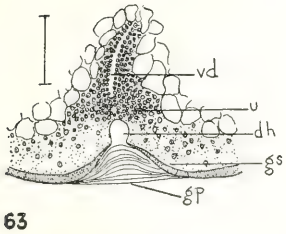
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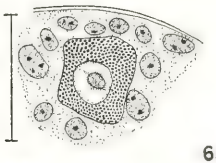
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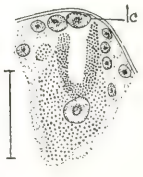
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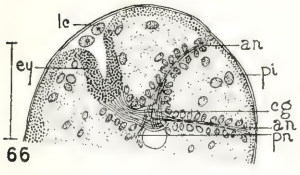
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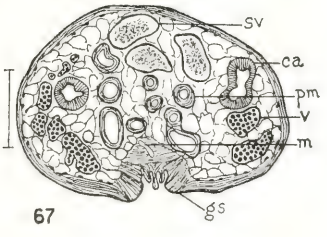
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PLATE VII

PLATE VIII

FIG. 68.—Sagittal section through genital complex of a specimen 2.5 x 0.72 mm. Scale 0.2 mm.

FIG. 69.—Cross section through genital sucker of a specimen 2.95 x 1.13 mm. Scale 0.1 mm.

FIG. 70.—Sagittal section of anterior end of a specimen 1.09 x 0.39 mm. Scale 0.2 mm.

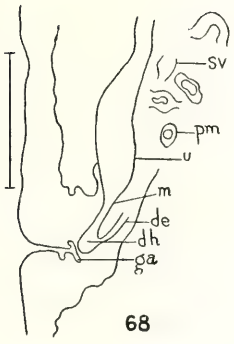
FIG. 71.—Sagittal section of anterior end of a specimen 2.46 x 0.63 mm. Scale 0.5 mm.

FIG. 72.—Sagittal section of anterior end of a specimen 6.0 x 2.75 mm. Scale 1.0 mm.

FIG. 73.—Sagittal section of anterior end of a specimen 9.0 x 2.75 mm. Scale 1.0 mm.

FIG. 74.—Cross section through genital sucker of a specimen 8.0 x 2.75 mm. Scale 0.1 mm.

FIG. 75.—Cross section through genital sucker of a specimen 4.0 x 1.15 mm. Scale 0.1 mm.



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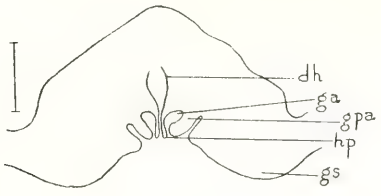

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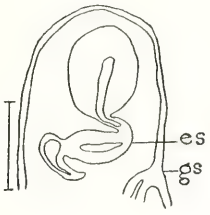

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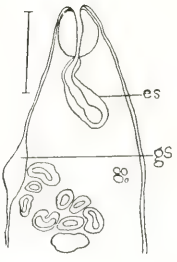

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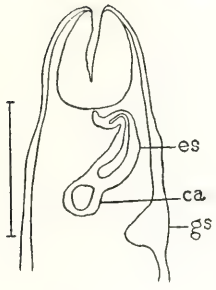

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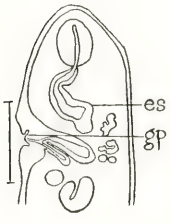

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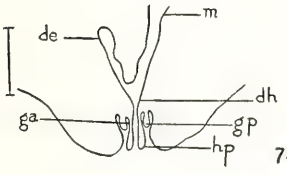

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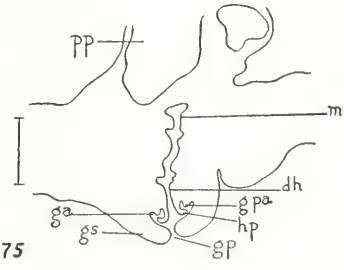

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PLATE VIII

PLATE IX

FIG. 76.—Sagittal section of a specimen of migration size, 2.46 x 0.65 mm. Scale 1.0 mm.

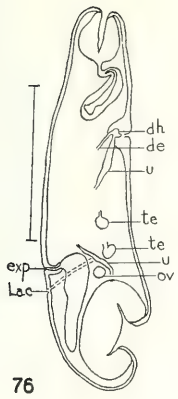
FIG. 77.—Sagittal section of a very young specimen. Scale 1.0 mm.

FIG. 78.—Sagittal section of a mature specimen 2.8 x 1.26 mm. Scale 1.0 mm.

FIG. 79.—Frontal section of a mature specimen 2.99 x 1.61 mm. Scale 1.0 mm.

FIG. 80.—Graphic reconstruction of a mature specimen. Scale 1.0 mm.

FIG. 81.—Sagittal section of a mature specimen. Scale 1.0 mm.



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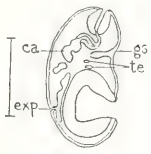

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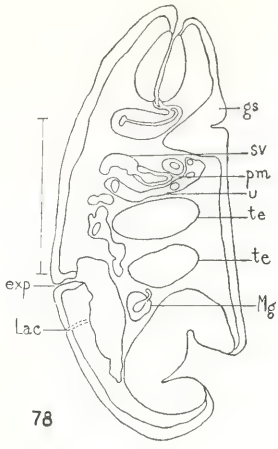

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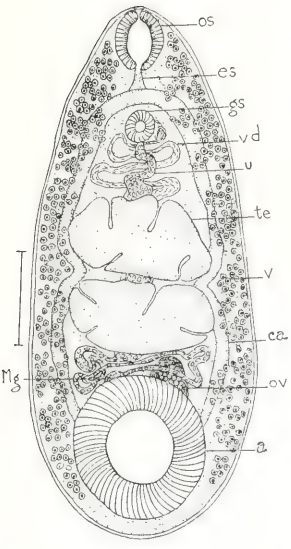

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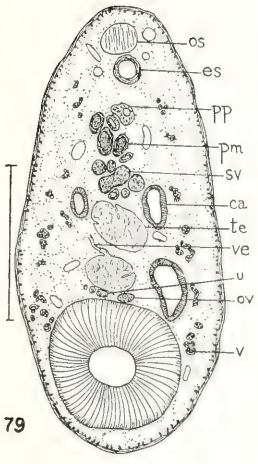

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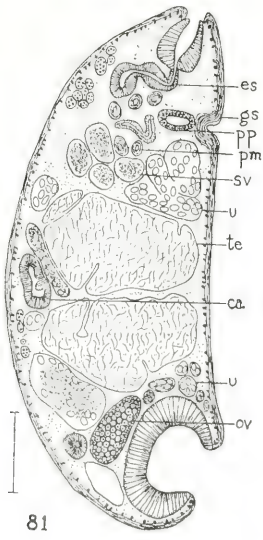

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PLATE IX

UNIVERSITY OF ILLINOIS BULLETIN
Vol. XXXIV September 29, 1936 No. 9

ILLINOIS BIOLOGICAL MONOGRAPHS
Vol. XIV, No. 4, Price $1.50

The Life History of *Cotylophoron cotylophorum* a trematode from ruminants

BY

HARRY JACKSON BENNETT

PUBLISHED BY THE UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

UNIVERSITY OF ILLINOIS BULLETIN
ISSUED TWICE A WEEK

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